

FIGURE 1. BOYCE THOMPSON INSTITUTE, EAST ELEVATION

Volume 1

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Number 1

## CONTRIBUTIONS

*from*

BOYCE THOMPSON INSTITUTE  
FOR PLANT RESEARCH, Inc.



Organization—Equipment—Dedication

Boyce Thompson Institute for Plant Research, Inc.  
1086 North Broadway, Yonkers, New York



205162

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BOYCE THOMPSON INSTITUTE FOR  
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1086 North Broadway  
Yonkers, N. Y.

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## PURPOSES OF THE INSTITUTE

Boyce Thompson Institute for Plant Research, Inc. is a membership corporation incorporated under the laws of the State of New York. Although the certificate of incorporation authorizes the carrying out of every phase of research on plants and the publication and dissemination of information related thereto, the purpose as conceived at present is fundamental research on practical plant problems and the publication of the results of such researches.

### ENDOWMENT

The Institute has been generously endowed by the founder, Colonel William Boyce Thompson.

### ADMINISTRATION

The Institute's charter provides for a Board of Directors, not to exceed eleven in number. This board is charged with the maintenance and care of the endowment and property of the Institute. The Board of Scientific Advisers are to advise the Director on the organization and management of the scientific work of the Institute and in the selection of the scientific staff.

### ORGANIZATION AND PRESENT SCOPE OF THE SCIENTIFIC WORK

The Division of Plant Physiology is in the direct charge of Doctors Crocker and Denny and with them are associated Mr. Davis, Doctor Reid, Mr. Morinaga, Miss Joseph and Mrs. Davis. Among the several projects being studied in this division are problems in plant propagation and the effects of various chemicals as well as hormones and endocrines, upon the rate, course of development and metabolism of plants.

The Division of Plant Pathology is in the direct charge of Doctor Kunkel and with him are associated Doctor Massey, Doctor Hartzell, Mr. Holmes, Miss Purdy and Miss Dobroscky. This division is giving much attention to the mosaic type of plant diseases including the yellows diseases of plants.

The Division of Biochemistry is under the direct charge of Mr. Arthur and Doctor Kraybill and with them are associated Mr. Popp, Mr. Webster, Doctor Davis and Mr. Gilbert. The major project of this division is the study of the effect of a great range of controlled environmental and nutritional conditions upon the metabolism and development of plants. The Institute is especially equipped for such studies.

The Division of Microchemistry is under the direct charge of Doctor Eckerson and associated with her are Doctor Goerrig and Miss Lampe. Besides developing new methods in microchemistry, this division has several other projects under way and is rendering much assistance on projects of other divisions.

The Division of Physical Chemistry is under the leadership of Doctor Youden and is devoting its attention to physical constants of plants. At present the work of this division is largely on the physical-chemical phases of the projects handled by other divisions.

The Division of Morphology is under the leadership of Doctor Pfeiffer. At present the work of this division is largely upon the anatomical and morphological phases of the projects handled by other divisions.

The lines of research mentioned above include only a few of the projects under investigation at present. The details of these and other problems will be described in the serial publications of the Institute mentioned later in this booklet.

While each project is under the leadership of one or another of the divisions, a number of the projects are being studied by several divisions. For instance, in the problem on the mosaic and yellows diseases of plants the physical chemist is handling the physical constants of diseased and healthy plants and determining the filterability of the viruses of these diseases, while the biochemists are studying the chemical constitution of the diseased and healthy plants and the microchemists are determining the localized chemical and anatomical differences between the diseased and healthy plants. An entomologist and protozoologist are investigating other phases of this project. This sort of coordinated many-sided attack is being used wherever it leads to surer and more rapid progress in the problem.

From this it will be seen that the scientific divisions mentioned above are more a classification on the basis of technic than on the subject matter covered.

#### FELLOWSHIPS AND TEMPORARY APPOINTMENTS

While the heads of the several divisions and a number of others of the scientific staff are on permanent appointments, several of the staff are temporary appointees. There are three classes of the latter: pre-doctorate fellows; post-doctorate fellows; and older investigators who have important problems well under way but need a year or two under good research conditions to complete their investigations.

The pre-doctorate fellows are selected from the most promising young investigators who are registered for doctor's degrees in the graduate schools of American universities. They spend one or two years at the Institute working out their theses on some phase of a

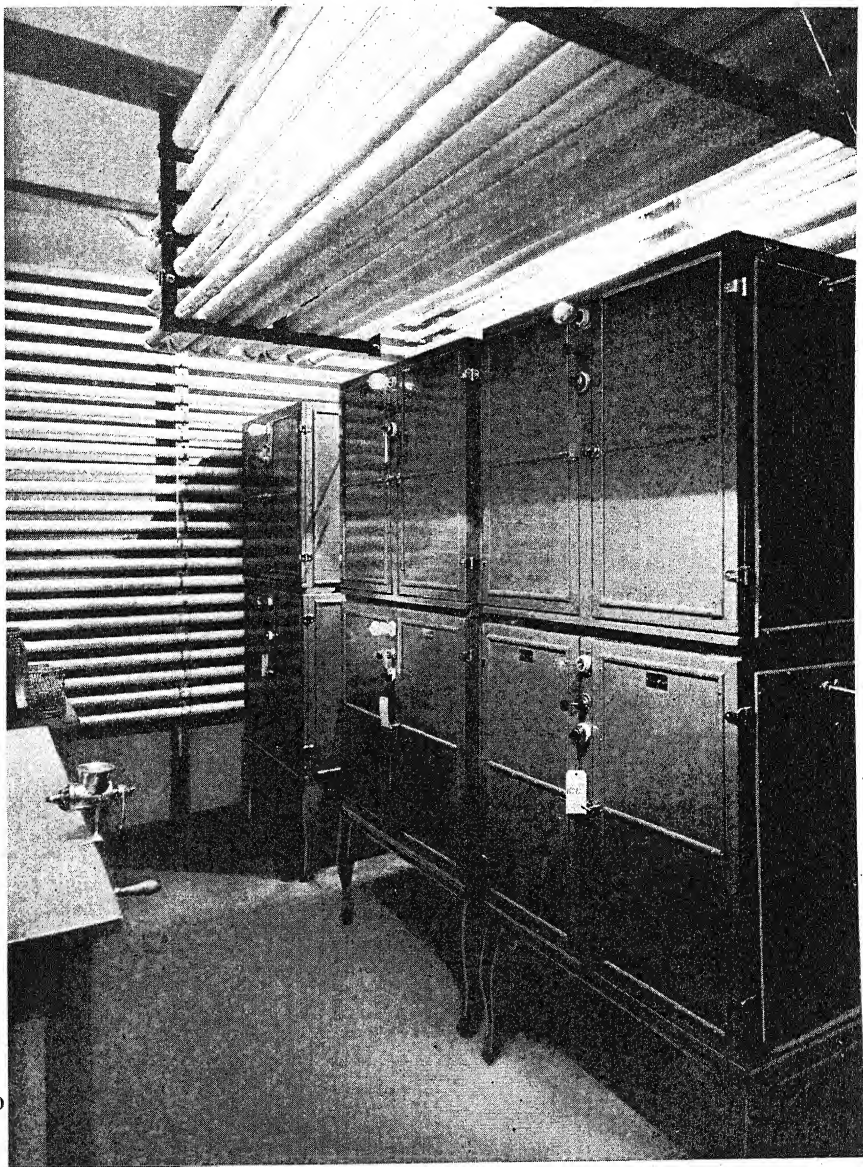


FIGURE 2. REFRIGERATION ROOM WITH TEMPERATURE CONTROL CHAMBERS



project being investigated at the Institute or upon a problem they have previously selected—the university accrediting this work for doctors' degrees.

The post-doctorate fellows are chosen on practically the same basis as the Biological Fellowships of the National Research Council.

The aim of these fellowships is not only to contribute to the knowledge of plants but to develop competent investigators in the field of plant science.

#### COOPERATION WITH OTHER INSTITUTIONS

The following is a list of those entirely supported by other organizations that have been or are now working at the Institute and making use of the advice of the staff and equipment of the laboratories:

Alice M. Anderson, M. A.—Development and germination of seeds of bluegrass—United States Department of Agriculture.

George H. Godfrey, Ph. D.—A study of some of the new fungicides and insecticides—New York City.

A. B. Massey, B. S.—Bean rust—Virginia Polytechnic Institute.

Dean P. W. Zimmerman, M. S.—Rooting cuttings of fruit trees—University of Maryland.

Such cooperation will be encouraged so far as the facilities of the Institute will permit and so far as the cooperation requested promises real advances in plant science.

#### PUBLICATIONS

Arrangements have been made for the prompt publication of the strictly scientific articles in standard botanical journals. Separates of these will be bound under covers of the Institute's own design and numbered serially as *Contributions* from the Institute. These will be arranged for binding in volumes of about six hundred pages each. A second series of *Professional Papers* will consist of separates of articles published in trade and technical journals. They will be arranged for binding in volumes similar to the *Contributions*. Both series of publications will serve for exchanges with other plant research institutions.

#### LABORATORIES AND EQUIPMENT

##### *Architectural and Structural Features of the Building*

The building is a highly specialized piece of construction, designed to meet unusual requirements. While architectural appearance was a secondary consideration the building is a well-balanced composition and designed to form a proper part of the ultimate group. The building

is of Colonial design with dark clinker brick exterior. The construction is of steel with concrete fireproofing and reinforced concrete floor systems. The finish is of sanitary simplicity, the appointments of the offices and laboratories are substantial and the equipment and fixtures of the best design. The present building is 247 feet long and 131 feet wide with the central building 50 feet by 117 feet, the north wing 50 by 73 feet and the remaining space occupied by the four main ranges of greenhouses, each of which is 20 feet by 117 feet. There is a 16 foot basement sub-structure under the buildings and greenhouses as well as under the court 73 feet long and 65 feet wide and under a roadway 247 feet long and 13 feet wide. The laboratories are housed in two stories (fig. 1).

### *Interior Distribution of Space*

In plan the building is designed with central corridors, with rooms located on either side. The corridors are placed off-centre so that the rooms on one side have a depth of 22 feet and on the other 17 feet. The corridor walls are permanent, while the partitions between the rooms, built of hollow tile, make possible a redistribution of space if necessary. The whole building may be subdivided, if required, into individual unit laboratories, which would total 84 in number.

### *Laboratory Services*

Since the laboratories require many pipes, conduits and ducts for their operation, a systematic arrangement was designed. All the piping is distributed horizontally along the exterior walls and connections are provided so that changes and redistribution may be easily arranged. The piping is all exposed and accessible. The service includes hot and cold water, gas, high pressure steam, compressed air, vacuum, drainage, refrigerated brine, distilled water, electric service (110 and 220 volts) and exhaust and ventilating ducts. The supply and exhaust air ducts are carried over the corridor ceilings. Each laboratory unit is provided with a floor drain in case of stoppage of sink drains and a shower head is placed immediately over each laboratory door, with a control valve at the side of the door for extinguishing fire in the clothing of a laboratory worker in case of accident. The ventilating system is operated from a positive pressure fan located in the basement, from which the air is conducted through masonry ducts supported above the corridors with grilles opening into each laboratory unit. Similar ducts for exhaust purposes lead to exhaust fans placed in the attic. The laboratory hoods are also connected into this exhaust system.

### *Mechanical Equipment*

The mechanical equipment located in the basement consists of three boilers which have a maximum capacity of 145 h.p. Two are used at low pressure for heating and the third for high pressure steam for power and other purposes. The boilers may be fired with coal, coke or fuel oil. In connection with the boilers there is a carbon dioxide plant, consisting of a system of four glazed tile towers with accompanying fans which move the flue gases from the boilers for scrubbing in the towers.

The basement also contains the ventilating plant, humidifying apparatus, compressors, vacuum pumps and two refrigerating plants, one of five ton and one of fifteen ton capacity, of the ammonia absorption type. Tanks for oil storage and bins for the storage of coal, coke and other materials are located under the driveway so that they may be filled directly from delivery trucks. There is a large reserve storage space.

### GREENHOUSES

The four ranges of greenhouses are of the steel rafter curved eave type and all have roof and side ventilators. Two of the ranges have wall ventilators in addition. The ranges run east and west and are located south of the main building. The range nearest the building is separated from it by an eight foot alley. The succeeding ranges are separated from each other by five foot alleys and each range toward the south is stepped down two and one-half feet. This arrangement avoids shading, both of the building and the greenhouses themselves. A central corridor that communicates with the first floor of the main building separates each range into two equal parts and each of these parts is further divided by a partition into two equal sized houses. This gives sixteen separate houses in all and makes possible a great range of controlled greenhouse conditions suitable for a variety of experimental work.

### SPECIAL CONTROLS

Provisions are made for the control of a number of environmental factors which affect the growth, form, structure and chemical constitution of plants. The controls as now established include temperature, constant condition light and dark rooms, auxiliary lighted greenhouses, spectral-glass greenhouses, humidified greenhouses and carbon dioxide plant.

### *Temperature Controls*

Provision has been made for accurate temperature control within very narrow limits yet of such a range that any required temperature

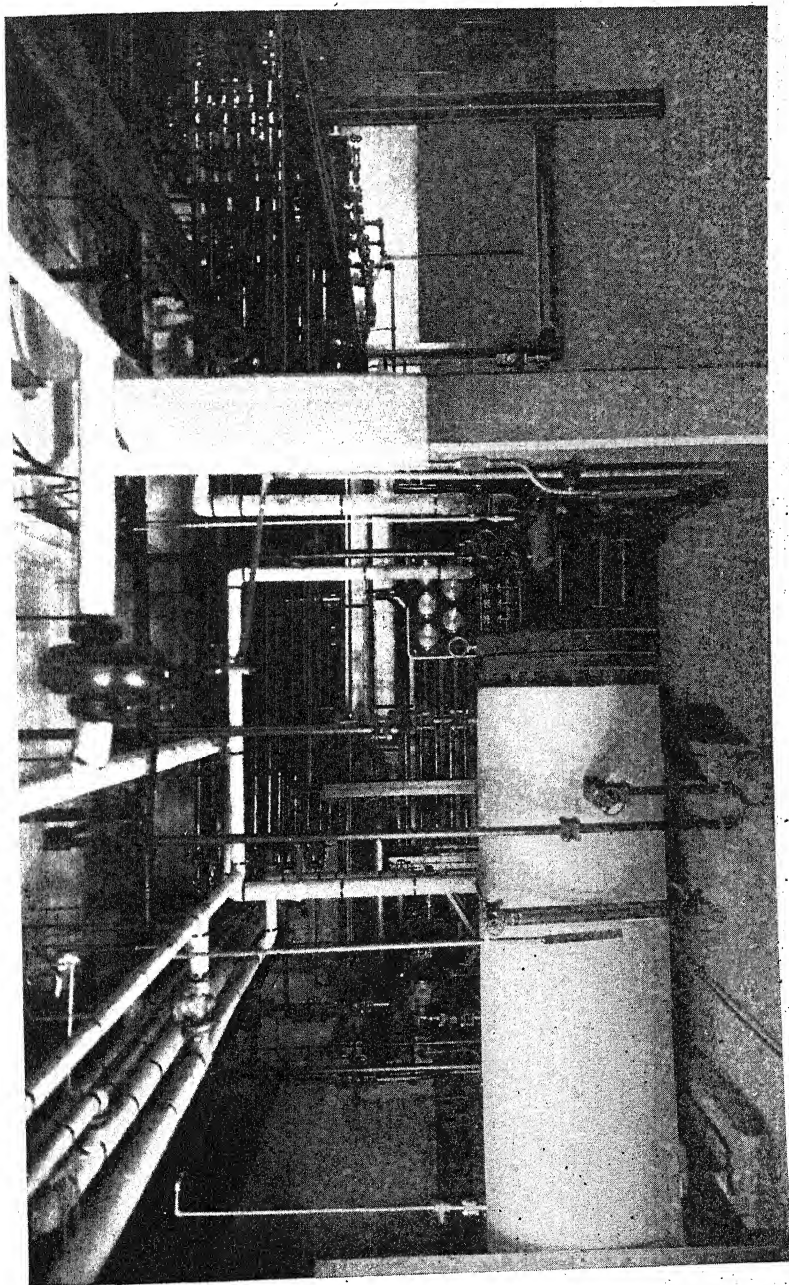


FIGURE 3. ONE OF THE REFRIGERATION UNITS

can be maintained. The range of controlled temperature is provided by using refrigeration for low and electrical heating for high temperatures, with a combination of these to produce intermediate temperatures. Accurately controlled temperatures above the room temperatures are obtained in laboratory ovens, heated electrically and controlled by electric thermo regulators. Accurately controlled temperatures below room temperatures are provided by similar ovens placed in the refrigeration room. The latter can be held at any temperature desired down to zero degrees Fahrenheit. This room is located on the first floor and is well insulated with heavy cork walls (fig. 2). Provision has also been made for a similar room above it on the second floor and below it in the basement, both of which may be equipped if additional cold storage room is required.

The two refrigeration plants are interconnected so that the unit suited to the load can be utilized (fig. 3). Calcium chloride brine at a temperature of zero degrees Fahrenheit is conveyed from the machines through well-insulated pipe lines to the refrigerator room and to many smaller box type refrigerators located in the laboratories of each department. Refrigerated brine is also available for use in the greenhouses. A part of the ammonia compressed by the machines is also used in cooling water for humidity and temperature controls in other parts of the building and for cooling potable water.

#### *Constant Condition Light and Dark Rooms*

The constant condition light and dark rooms are located in the basement under the first greenhouse. They consist of two adjoining rooms, each eleven feet square, connected by a short, closed corridor to facilitate transfer from one room to the other with minimum interference with conditions. The light room is illuminated by twenty-five one-thousand watt lights suspended from the ceiling. Temperature is controlled to within one degree and humidity to within two percent in both rooms by partial or complete re-circulation of the air through a spray tank maintained at a constant saturation temperature, or dew point. Temperatures can be held in both rooms as low as ever obtains in nature during the growing season, or can be raised to simulate the hottest days of summer. Light intensity can be decreased by turning off some of the lamps, which, owing to proper spacing and switching arrangement, does not affect equal distribution of light. Intensity in the constant light room is of the order of four hundred foot candles while the maximum of sunlight at noon in June is of the order of 10,000 foot candles. When one considers that ten foot candles is an unusually high intensity for office lighting he can appreciate the visual brightness within this room (fig. 4).

The system of lighting is sufficiently flexible to make possible

considerable increases in intensity. Since tungsten lamps are much richer than sunlight in the heat rays of the spectrum, a ray filter, consisting of a sheet of plate glass over which water is continuously circulated, is interposed between the lights and the growing plants in order to protect against excessive heating. Provisions are also made for introducing mercury vapor lamps to supplement the tungsten lamps which are poor in blue and violet rays.

#### *Auxiliary Lighted Greenhouses*

Two of the greenhouses are equipped for supplementing daylight by artificial light at night. The equipment consists of a large gantry crane electrically driven so it can be easily moved over the greenhouse at night and be removed in the day time. The crane carries forty-eight one-thousand watt lights, so spaced as to give uniform intensity over the center area of the greenhouses. This enables the investigator to lengthen the daily period of natural illumination at will, with an intensity of light amounting to four or five hundred foot candles (figs. 5 and 6).

#### *Spectral-glass Greenhouses*

A series of five spectral-glass houses makes it possible to test the effects, upon the development of plants, of eliminating various portions of the sun's rays. House number one is covered with ordinary greenhouse glass which absorbs the shorter ultra-violet rays; number two, with glass that transmits all the visible rays and practically all the ultra-violet; number three, with glass that absorbs all the ultra-violet but transmits all the visible rays; number four, with glass that absorbs the ultra-violet and blue but transmits all the longer rays; and finally, number five, with glass that absorbs the ultra-violet, violet, blue and most of the green but transmits the rest of the green and the yellow and red. These houses make possible a study of the effect of the quality of the light upon plant development so far as spectral glass now available permits such a study (fig. 7).

#### *Humidified Greenhouses*

A range of greenhouses is equipped with the necessary machinery for controlling humidity along with temperature. This consists of a system of metal air delivery ducts which bring humidified air from a humidifier in the basement to the center of each greenhouse, and a similar system of ducts which return the air to the humidifier. Air is saturated in the tank with water vapor at a temperature which is held constant and is then heated by steam to the desired greenhouse temperature as it rises through the delivery ducts. Air inside each

greenhouse in the humidified range is recirculated through the humidifier every two minutes. Humidity is controlled to within two percent and temperature to one degree (figs. 8 and 9).

### *Carbon Dioxide Plant*

The carbon dioxide plant as now established at the Institute consists of four towers made of glazed tile, a pump for circulating sodium carbonate liquor and a fan for moving flue gas produced in the boilers from the combustion of coke, coal or fuel oil. In the first two towers the gas is scrubbed with water and in the second two with sodium carbonate solution. The gas is then driven directly to the greenhouses to be used for increasing the concentration of carbon dioxide in the atmosphere of the greenhouse. Some injuries are produced on certain plants by carbon dioxide gas obtained in this way, as it is not entirely free from noxious gases. Additional towers where gases can be scrubbed with acid or perhaps other reagents are now being installed (fig. 10).

The aim is to determine the concentration of carbon dioxide that is best for the growth of various plants, the best daily period for maintaining this concentration, the best method of purifying the flue gas and the possibility of commercial application. When one realizes that more than forty percent of the dry weight of the plant is carbon and that this carbon is all obtained from the carbon dioxide of the air, he appreciates the importance of such studies.

### *Central Temperature, Humidity and Radiant Energy Recording Station*

The temperatures and humidities of the greenhouses, spectral-glass houses, constant condition rooms and the constant temperature chambers in the refrigeration room are recorded as desired at a central station. This is accomplished by electrical resistance thermometers in the several rooms or chambers which are connected by wires to the recording apparatus at the central station. The daily solar radiant energy is also recorded at this station by means of a pyroheliometer.

### LIBRARY

The nucleus of the collection was formed in 1922 when the Director purchased the major portion of the library of the late Philippe Van Tieghem of Paris. At this time he also personally inspected the book stocks of all the important German, English and French dealers and due to post-war conditions was able to secure many complete sets of periodicals.

The files of the United States Department of Agriculture and the state agricultural experiment station publications are nearly complete.

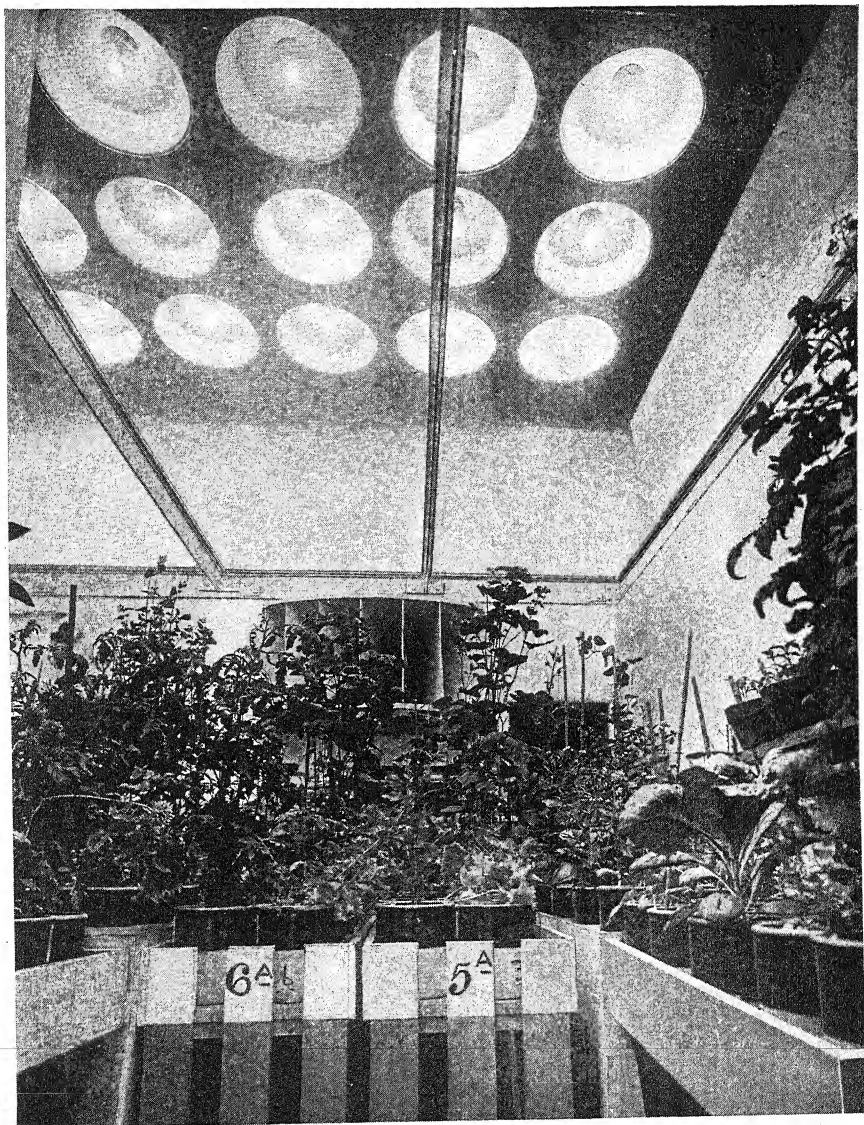


FIGURE 4. CONSTANT CONDITION LIGHT ROOM



The foundation for the agricultural literature was made by the purchase of the library of the late Dr. F. W. Woll of the University of Wisconsin and the University of California.

In all there are about 10,000 volumes. These are arranged according to the Library of Congress classification and Library of Congress cards are used in the cataloging wherever available. One hundred and seventy-five periodicals, exclusive of public documents, are currently received and nearly one-half of these are complete from their first issue. A trained librarian is in charge.

#### PHOTOGRAPHIC EQUIPMENT

There is a completely equipped modern photographic department consisting of a photograph room and two adjoining dark rooms for developing and printing. Apparatus is provided for photography, microphotography and moving pictures.

#### CHARTER OF THE INSTITUTE

Boyce Thompson Institute for Plant Research, Inc. is incorporated under the Membership Corporation Law of the State of New York. The following, excepting notarial and other certificates, is a true copy of the certificate of incorporation filed October 28th, 1924 in the office of the Secretary of State of the State of New York and in the office of the County Clerk of Westchester County, N. Y.

#### THE UNIVERSITY OF THE STATE OF NEW YORK

STATE OF NEW YORK }  
COUNTY OF ALBANY } SS.

Pursuant to the provisions of section 59 of the Education Law the Regents of the University of the State of New York do hereby consent to the filing of the annexed certificate of incorporation of "Boyce Thompson Institute for Plant Research, Inc." as a membership corporation under and pursuant to the provisions of the Membership Corporation Law.

IN WITNESS WHEREOF, I, Frank P. Graves,  
President of the University of the State of  
New York and Commissioner of Edu-  
cation, do hereunto set my hand and  
affix the seal of the State Education  
Department at the City of Albany, this  
28th day of October, 1924.

(SEAL)  
The University of  
The State of New York

FRANK P. GRAVES  
President of the University of the State of  
New York and Commissioner of Edu-  
cation.

## CERTIFICATE OF INCORPORATION

—OF—

## BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.

We, the undersigned, all being of full age and all except one citizens of the United States, and a majority of us being residents of the State of New York, desiring to form a corporation pursuant to Article 3 of the Membership Corporation Law of the State of New York, do hereby certify as follows:

FIRST: The name of the proposed corporation is BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.

SECOND: The object for which the corporation is to be formed is to make, institute, conduct and carry out all and every manner and kind of scientific agricultural, horticultural, biological or metallurgical experiment, research, study and investigation, and in any other way to assist in improving and developing plant and animal life, and to publish or otherwise disseminate or communicate information or advice with regard thereto. It shall be within the purposes of said corporation to use any means to those ends which from time to time shall seem to it expedient, including the establishment and maintenance of any activities, agencies and institutions, trust, fellowship or scholarship funds appropriate thereto, and the aid of any such activities, agencies or institutions already established, or which may hereafter be established. The corporation shall have power to acquire, by grant, gift, devise or bequest, either absolutely or in trust, and to hold and to dispose of, such property, real or personal, as the purposes of the corporation shall require, without limitation as to amount or value, except such limitation, if any, as is now or may hereafter be imposed by the laws of the State of New York; to apply for, acquire, and own any patent or patent rights, and to sell, lease or license the use of the same; to accept and administer in trust all property, real or personal, for any purpose within the objects of the corporation, and to prescribe, by by-laws or otherwise, the terms and conditions upon which property, real or personal, shall be acquired or received by said corporation.

By way of amplification and not by way of limitation of its powers, it shall further have power to build, purchase, improve, enlarge, equip and maintain laboratories and other buildings in the City of Yonkers, in the State of New York, and elsewhere, necessary or appropriate for its work; to own and operate land and buildings for the study of plants or for any other of its purposes; to furnish treatment for plant diseases, and to provide and maintain all necessary equipment therefor; to conduct and assist such scientific experiments or investigations upon plants as may be necessary or proper for carrying on its work of

research; to appoint committees of experts to direct special lines of research; to aid, cooperate with or endow individuals, associations or corporations engaged in similar work or in preparation therefor, within the United States of America or elsewhere; to aid or cooperate with investigators in its own laboratories or elsewhere; to collect statistics and information, and to publish and distribute documents, reports and periodicals; to carry on such educational works along the lines of its corporate purposes as it may deem wise, and to conduct lectures and hold meetings; to erect and maintain museums, and in general to do and perform all things necessary or convenient for the objects of the corporation, or any of them. Such activities are to be carried on for the advancement of human knowledge, and no part of its earnings or net income is to enure to the benefit of any stockholder.

Upon the dissolution of the corporation any property, real or personal, of the corporation then remaining after the payment and discharge of its obligations and liabilities, shall be transferred, conveyed, delivered and paid over to such institution or institutions upon such terms and conditions, and in such amounts and proportions as the directors shall determine, to be used by the institution or institutions receiving the same for the same purposes set forth herein or similar purposes, and to be known as the *BOYCE THOMPSON FUND* or *FUNDS FOR PLANT RESEARCH*, and no member of the corporation shall be entitled to receive any part of said property.

**THIRD:** The territory in which its operations are to be principally conducted is the State of New York, but it is not to be restricted thereto.

**FOURTH:** The town, village or city in which its principal office is to be located is the City of Yonkers.

**FIFTH:** The number of its directors is eleven.

**SIXTH:** The names and places of residence of the persons to be its directors until its first annual meeting are as follows:

<i>Name</i>	<i>Address</i>
William Boyce Thompson,	1061 North Broadway, Yonkers, N. Y.
Margaret T. Schulze,	300 Park Avenue, New York, N. Y.
Theodore Schulze,	300 Park Avenue, New York, N. Y.
William Crocker,	1086 North Broadway, Yonkers, N. Y.
John M. Coulter,	University of Chicago, Chicago, Ill.
Lewis R. Jones,	Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin.
Frederick J. Pope,	1086 North Broadway, Yonkers, N. Y.
Caleb C. Dula,	1109 North Broadway, Yonkers, N. Y.
Frederick H. Ecker,	c-o Metropolitan Life Insurance Co., 1 Madison Avenue, New York, N. Y.

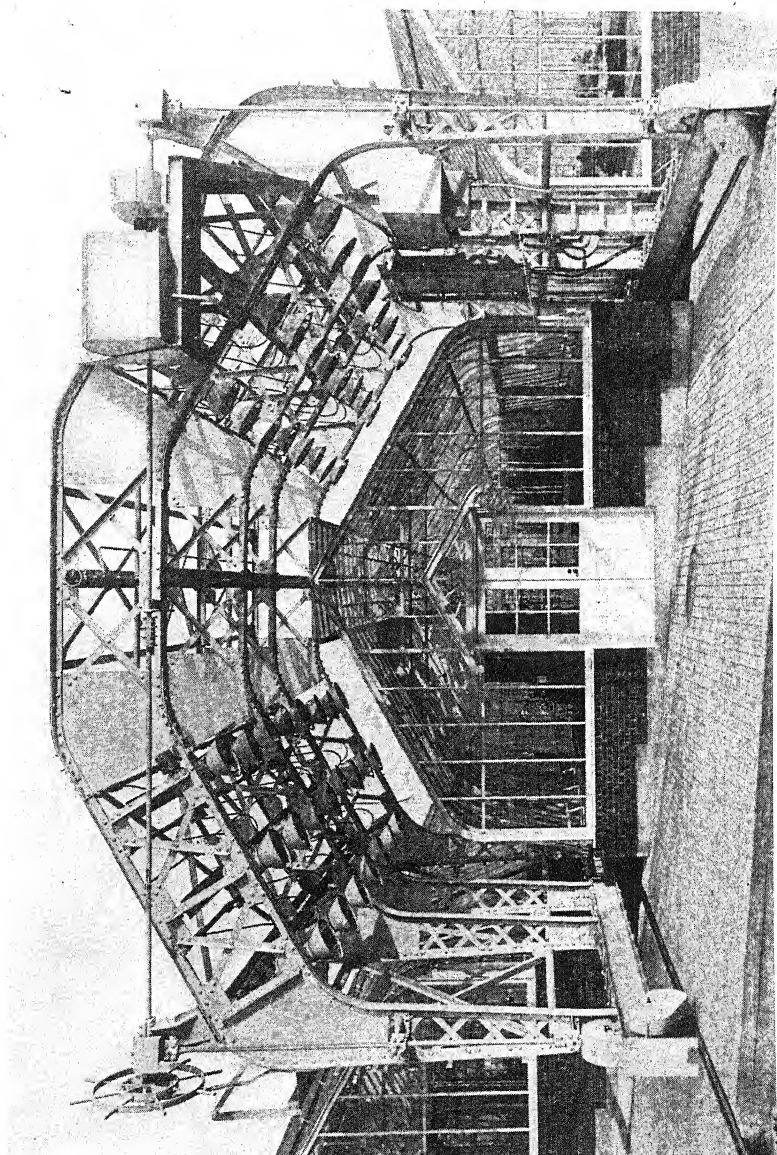


FIGURE 5. GANTRY CRANE FOR ILLUMINATING GREENHOUSES

<i>Name</i>	<i>Address</i>
Raymond F. Bacon,	Pelham, N. Y.
Charles F. Ayer,	1 Slocum Street, New Rochelle, N. Y.

IN WITNESS WHEREOF we have herunto signed our names and affixed our seals this 25th day of October, 1924.

William Boyce Thompson  
Margaret Thompson Schulze  
Theodore Schulze  
William Crocker  
Frederick John Pope  
Caleb C. Dula  
Charles F. Ayer

The foregoing certificate of incorporation of BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC. is hereby approved.

DATED, October 27th, 1924.

VERNON M. DAVIS,  
Justice of the Supreme Court.

(Notarial certificates, etc., omitted.)

## ADDRESSES

*At Official Opening of the*

# BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, Inc.

*Wednesday, September 24, 1924*

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PROFESSOR JOHN M. COULTER, *Presiding,*  
*Professor of Botany, University of Chicago, Chicago, Illinois*

It is my privilege on this occasion to act as spokesman for the Boyce Thompson Institute for Plant Research, and for its Board of Directors. Your presence here bespeaks your interest in this great enterprise, an interest which we trust will increase as time goes on, because justified by the results. We greet you, therefore, as friends and well-wishers. Become acquainted with the opportunities, and tell your friends of the great project that is now under way, and also of its significance in public service.

In my judgment, this Institute stands for an epoch in the history of botanical science in this country. Botany has come to be recognized as a great national asset, dealing with problems that are of fundamental service to the whole population. It has been said that the science of Medicine serves the unfortunate minority, while the science of Botany serves every one. The public is beginning to realize our general dependence upon plants and their products, and the relation of botanical research to our better cultivation of these resources. It is this point of view that has rehabilitated Botany in public estimation as a science of first importance to human welfare. It is very timely, therefore, that an Institute for Plant Research should be established, to carry forward work so fundamental to the interests of mankind, and Botany is to be congratulated that the founder of this Institute has had such a prophetic vision of the future.

Looking now to the future, there are certain general tendencies that can be projected and thus enable us to adjust ourselves to the inevitable direction of progress. I am confident that a progressive spirit will animate the Boyce Thompson Institute, a spirit that is dynamic rather than static, and it is this feeling that led me to speak of this occasion as representing an epoch in the history of Botany. Botanical research is going on in scattered institutions throughout the whole country, but in practically every case with handicaps in freedom

of opportunity and equipment. Such a center of research as we are formally opening today furnishes the necessary freedom of opportunity and equipment, and cooperates with all other institutions to their mutual advantage. In other words, it represents a synthesis of botanical research, helping to coordinate the segregated units.

I wish to name three tendencies in Botany which seem to me to be significant of the future.

One growing tendency is to attack problems that are fundamental in connection with some important practice. The outstanding illustration, of course, is the increasing attention given to the problems that underlie agriculture; but there are many other important practices also which are bedded in botanical investigation.

A second tendency is an increasing realization of the fact that botanical problems are synthetic. Around each bit of investigation, with its single point of view and single method of attack, there is developing a perspective of other points of view and other methods of attack.

A third tendency is the growing recognition of the fact that structures are not static that is, inevitable to their last detail; that what we see are responses to varying conditions. This means the development of experimental control, to understand the *behavior* of Nature rather than to observe simply the random results of her activities.

Of course the most important ideal of Botany, as of all sciences, is to extend the boundaries of human knowledge, the goal being to understand Nature. This ideal includes no thought of making Nature a servant to minister to our needs. To know Nature simply because it is wonderful and worth knowing is what it means. Such investigation is like the exploration of an unknown continent. Every advance into the new territory impresses us with the fact that it is far more extensive than we had dreamed. Every trail is worth following because it means additional knowledge. Some trails may lead to rich farm lands or gold mines, but in exploration these are only incidents. To understand the new country, all trails must be followed and mapped.

To summarize the whole situation, I would say that the ideals of this Institute are (1) to understand plant life, that the boundaries of human knowledge may be extended, and man may live in an ever-widening horizon; and (2) to apply this knowledge to the service of man, that his life may be fuller of opportunity.

It is a great mission, and may the future justify the thought and hope that this Institute represents.

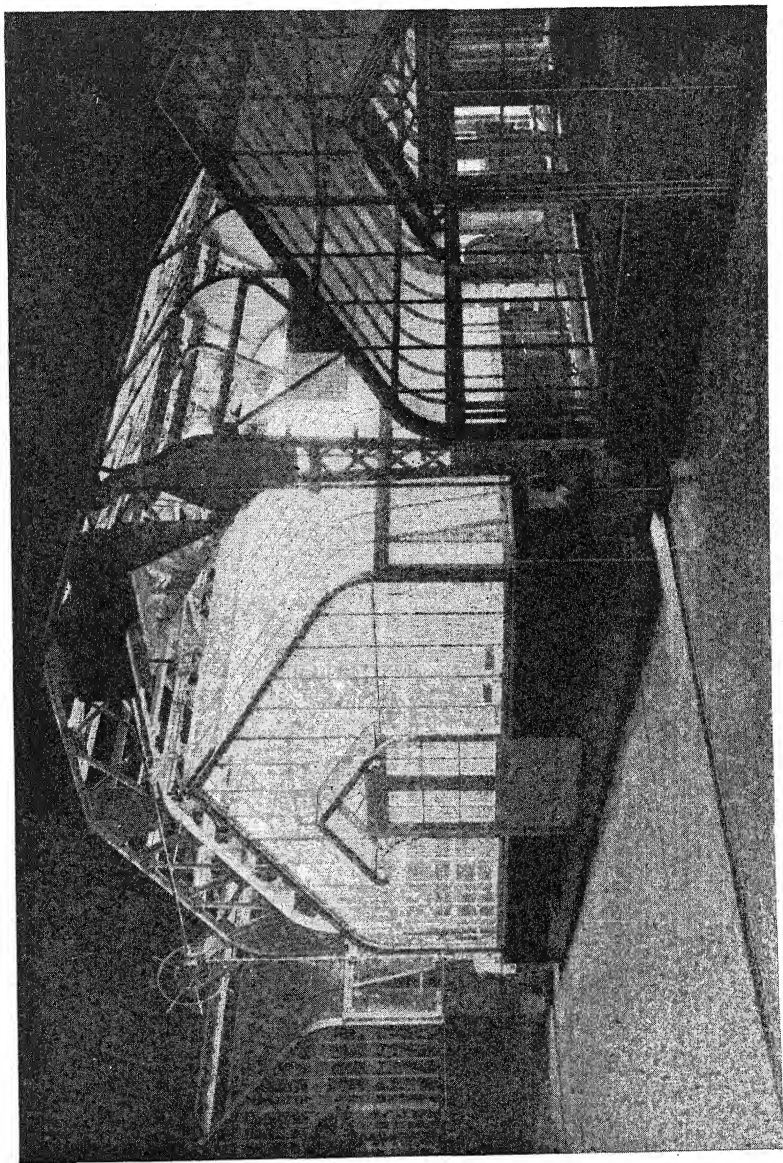


FIGURE 6. GREENHOUSE ILLUMINATED AT NIGHT



AIMS OF BOYCE THOMPSON INSTITUTE  
FOR PLANT RESEARCH.

DOCTOR WILLIAM CROCKER, Director.

Mr. Chairman, neighbors, fellow scientists and friends of Boyce Thompson Institute for Plant Research.

## MAN'S DEPENDENCE UPON PLANTS

On this occasion it is well to stop for a moment to consider the great importance of plants to ourselves. Such consideration will throw much light on the significance and aim of the Institute we are dedicating today.

The energy of the gas that runs our automobiles, of the coal that heats our homes and furnishes power for our factories and railroads and of the food that warms our bodies and gives energy for our physical and mental activities is all fixed by the green leaf of the plant from the sunlight. The total of the energy thus fixed and deposited in coal, petroleum and other organic materials of the earth amounts to millions of trillions of calories—numbers quite beyond human comprehension.

Human beings and animals are just as dependent upon plants for the materials out of which their bodies are made as they are for the energy just mentioned. The sugars, out of which all organic materials of living organisms are made, are manufactured by plants in the same chemical reaction in the green leaf that fixes the energy from the sunlight. The green leaf of the plant is the world's most wonderful chemical factory and powerhouse!

The proteins or albumin-like bodies—that are always the main constituents of living matter—can be made without limit only by plants. Several of the score of building-stones, or amino acids, that make up proteins can not be made by animals but must be made and furnished to them by plants.

We all hear much about vitamins these days. They are necessary in many ways for the development and normal functioning of the animal and human body. They are preventers of several human disorders. One of the latest discovered is even necessary for reproduction in man and animal. So far as evidence now shows the vitamins are made mainly and probably exclusively by plants.

If I may be pardoned for venturing still farther into a scientific field not my own, I will suggest that the outstanding advance in animal physiology during the last two decades has been in the knowledge of two groups of substances that to a great degree control the development and regulate the functions of the human and animal body. These are the vitamins, which we have just discussed, and endocrines, or secretions of ductless glands. In a few years "endo-

crines" will be as much an everyday newspaper term as is "vitamines" today. We are all acquainted with one of the endocrines, insulin, the newly discovered and almost miraculous remedy for the formerly baffling disease, diabetes. Knowledge of these substances is rapidly giving man control of human development and in part, laying scientific foundations for a new medicine. It is more than interesting that a substance very similar to, if not identical with insulin, is abundant in plants.

Are plants in part, or in the main, the ultimate source of endocrines for animals, as they are the ultimate source of vitamins?

Again the abundance of vitamins and the presence of endocrine-like substances in plants raises the question of the function of these bodies in plants themselves. This is a field of plant science of which we know nothing at present and a field the mastery of which may give unexpected control of plant development.

This brief enumeration of facts and unsolved problems shows the intimate and many-sided dependence of man upon plants. No science is more fundamental to life and more immediately and multifariously practical than plant science. In plant science are enough unsolved riddles to tax the best scientific genius for decades to come. Doctor Haldane, an English biochemist, has recently written a fascinating and prophetic little book, *Daedalus*, based upon recent advances in animal physiology and biochemistry. Because of the control these advances are giving man over human development, disease and activity, he pictures the biologist as the miracle man of the future.

### ECONOMIC SIGNIFICANCE OF PLANTS

Now let us look in a very practical way at the significance of plants and plant research. In 1919 the total raw agricultural products in the United States were worth about 24 billion dollars—more than enough to cancel our entire national debt. In 1922, with the low prices, these products were worth fourteen and one-half billion dollars. Using round numbers, two-thirds of this was used as plant material and one-third as animal. The animal material was plant before being converted into animal, at a tremendous loss of energy and material. A billion dollars is a lot of money. Had one begun saving at the rate of a dollar a minute with the beginning of the Christian era he would now be a little beyond the billion dollar mark. These facts give some conception of the enormous economic significance of plant agriculture in the United States. The great diversity of crops, of soils and of climatic conditions, under which these crops are grown, add to the multiplicity and difficulty of the problems arising in this field.

After the Civil War the rich virgin lands of central and western United States were rapidly put under cultivation. This was made possible by the development of transportation facilities and improvement of farm machinery. As a consequence, for a number of decades, agricultural production increased faster than the population and this country became a great exporter of agricultural products. But there was a limit to new land, so there came a time when the population increased faster than agricultural production. As nearly as can be determined, as a matter of averages, this focal date is 1906.

#### THE FUTURE POPULATION AND FOOD SUPPLY OF THE UNITED STATES

At any rate we are now living in a period when the population in the United States is increasing faster than agricultural production and our exportable excess is decreasing. If we may judge from statistics on growth of population and agricultural production, our consumption will equal our production in fifteen or twenty years. In fifty years our population will be one hundred ninety-five millions. This will demand about seventy-five percent more food than we produced annually during the period 1911 to 1921.

After fifty years our population will continue to grow and demands for food will continue to increase. We can not meet this ever-growing demand with additional rich acres. The rich acres of this country are now largely under cultivation. Greater production must be brought about mainly by increasing yields on fertile lands and by making poor lands more productive. As a foundation for such increases, years of basic scientific research will be required on propagation from seeds and cuttings, cultural methods, use of fertilizers, production of new and better breeds of plants, control of plant diseases and insect pests, and methods of harvesting, shipping and storing.

#### NEED OF CONTROLLED CONDITIONS FOR PLANT RESEARCH

Most excellent research on plants has long been under way in the United States and many other countries, but there have been very definite needs of strengthening this research in certain important directions.

There has been the need of controlling accurately and on a rather large scale for the entire life of the plants all of their growth conditions—light (quality, intensity and daily duration), temperature, humidity, carbon dioxide concentration of the air, as well as soil conditions. In your inspection this morning you saw our attempt at working out such controls on a considerable scale. In spite of making use of the best engineering skill available today in the construction of the control greenhouses and chambers we find that much research

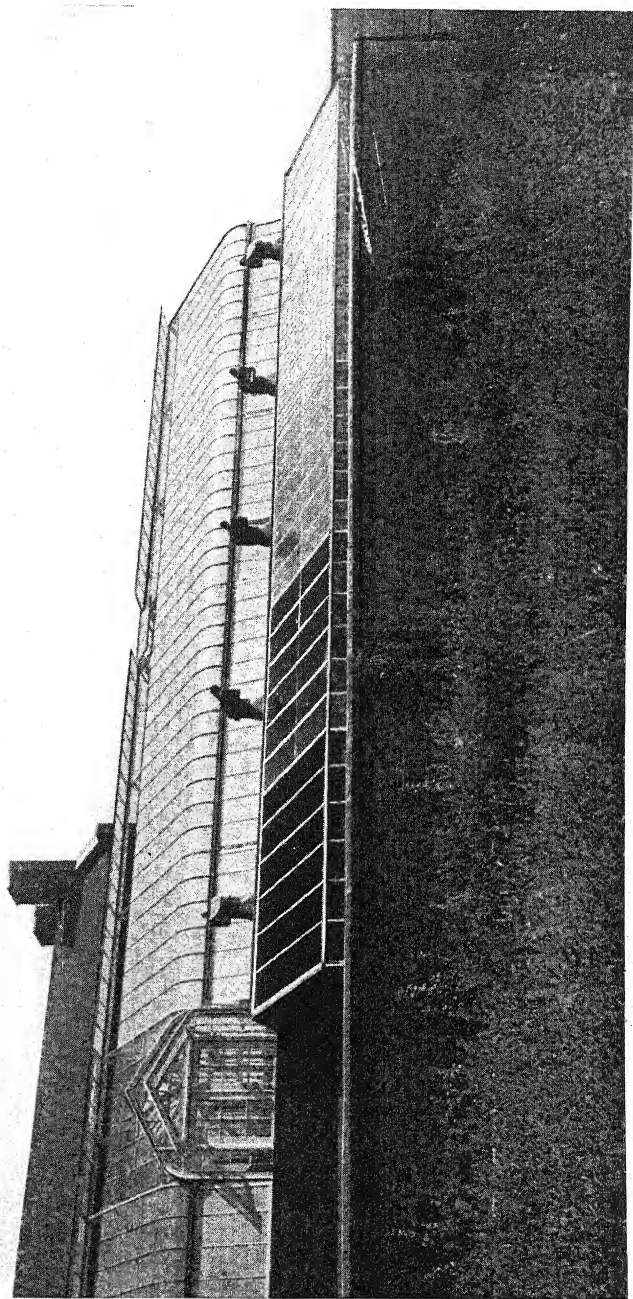


FIGURE 7. SPECTRAL-GLASS GREENHOUSES

must be done in perfecting certain of these controls along with the years of research necessary to establishing the laws of plant development that such controls make possible. Because of lack of engineering knowledge and mechanical development we have thought it best to install originally only a proportion of the controls we expect finally to put into operation. We hope that advances in mechanics and discoveries made with the present controls will enable us to improve the later installations and build them at less cost.

#### FUNDAMENTAL RESEARCH AND PLANT PRODUCTION

The quickest way to solve, finally, many of the practical plant problems is a thorough-going long-time investigation aimed at the establishment of basic principles. One of the weaknesses of democracy is the fact that it is hard to get legislators to see the desirability of appropriating funds for such research. If the legislators do see the desirability they may be unable to convince their constituents of this necessity. Our excellent government and state institutions as well as other plant research institutions in the United States have been in need of an institute or institutes where some of their basic long-time problems can be taken and worked out with adequate equipment and with the aid of a well-rounded able scientific staff. Boyce Thompson Institute hopes to aid in supplying this need. If I may judge from the interest that other institutions have shown, and from the number of young agricultural and botanical investigators desiring to work here, the present capacity of our building will soon be overtaxed and we shall need to think of building others of the additional three units that are to complete the proposed quadrangle of the Institute.

#### EQUIPMENT AND ORGANIZATION FOR RESEARCH

Scientific research is of necessity inefficient, for it is a matter of exploring an unknown country where there is only the merest hypothetical suggestions of where the great treasures lie. In some institutions in the United States plant research has been made even more inefficient because of inadequate equipment and because the scientific workers are compelled to do work that ought to be done by engineers, mechanics, stenographers, librarians and even laboratory boys. Scientists generally do these other lines of work very poorly and at great cost. We are trying to build up an organization where the plant investigator can devote all his time to the subject matter and technic of his field. In short, we are trying to make our scientific work as efficient as possible considering its inherent nature. We are finding that such an organization requires about one other worker to each plant scientist.

## COOPERATIVE ATTACK ON PLANT PROBLEMS

The unsolved problems in our sciences, more especially biological sciences, are becoming more and more complex and difficult. In the main the easier problems were solved first. Many of these problems are so complex that one man can not acquire enough knowledge or learn enough technic to solve them. Several scientists of different training and technic must combine for their final and quick solution.

I can best illustrate this need of concerted all-sided attack on modern scientific problems by a quotation from a little pamphlet, "The Future Independence and Progress of American Medicine in the Age of Chemistry" written by a committee of the American Chemical Society.

"When it became clear during the recent war that poisonous gas was to constitute an important munition, our country called to its service a great group of its ablest research chemists to provide efficient means of defense and to solve those problems of production which would provide our field forces with an ample supply of this new weapon.

"Not to the professional inventor nor to the accident of hap-hazard discovery was this grave responsibility assigned, but to the trained workers in systematic research. Nor were these men asked to undertake this vital work in the seclusion and isolation of their respective laboratories, but they were assembled at the American University Experiment Station on the outskirts of the City of Washington, under one roof as it were, where, by daily, nay hourly conference, utmost speed could be secured in the solution of those problems on which the question of life and death so closely hung.

"But these chemists found that they alone were inadequate for the task. To supplement their special skill and knowledge there were added to the staff pharmacologists and experimental pathologists.

"Through the combined efforts of these groups, working in closest association and provided with ample facilities for research, results were accomplished with a speed and certainty which amazed all. The paths to agencies for both defence and offense were clearly pointed out and large scale production quickly followed."

The necessity for this manner of approach to large scientific problems is well illustrated in plant science by the attack the Institute is making upon the puzzling and destructive group of plant diseases included under the names of mosaic diseases, virus diseases and yellows. The attack is headed by a plant pathologist. As the diseases are largely carried by insects, an entomologist is associated with him.

Unlike the other two groups of plant diseases caused by bacteria on one hand and by fungi on the other, the causative agent of mosaic and yellows diseases of plants is not known. There is, however, marked

similarity between the insect transmission of some of these plant diseases and insect transmission of yellow fever, malaria and sleeping sickness, diseases of man caused by microscopic animals called protozoans. This and other evidence raises the suspicion that some of the yellows and mosaic diseases of plants may be caused by protozoans. Consequently, a protozoologist has been associated with the plant pathologist to investigate this possibility. Chemists of various training, a microchemist, a plant physiologist and others are included in the team working on this problem.

We propose to use this method of coordinated all-sided attack on many of our projects so far as it will make for most rapid advance and not interfere unduly with individual initiative. Our permanent staff is selected with this in mind.

#### FELLOWSHIPS

Besides the attempt to organize a well-rounded able permanent staff we propose to establish a system of advanced research fellowships for the purpose of giving able young men an opportunity to develop further their research ability under the guidance of the permanent staff and with the use of adequate equipment. It is believed that a continual flow of temporary younger appointees through the Institute will avoid stagnation and fossilization. This is a condition that frequently occurs in research institutions which lack the rejuvenating influence that comes from association with alert and ambitious young minds. Cooperation with practical and scientific plant research institutions ought to act also as an anti-fossilizer.

#### INTIMATE RELATION BETWEEN SCIENCE AND PRACTICE

In the study of plants, science and practice are capable of most intimate association with reciprocal benefits; so our avowed aim, fundamental research on practical plant problems, should offer equal promise of advance in plant science and of improvement in practice.

Thus I have presented briefly, and I fear, in a rather fragmentary way, the aims of the Institute we are dedicating today.

In closing, may I say that my co-workers and I have been greatly inspired by the friendly and helpful interest shown by you here today as well as in the past. Our greatest hope is that we shall be able to lay the proper foundations for the realization of the great vision of future usefulness that our far-sighted and generous founder had, when he established and handsomely endowed Boyce Thompson Institute for Plant Research.

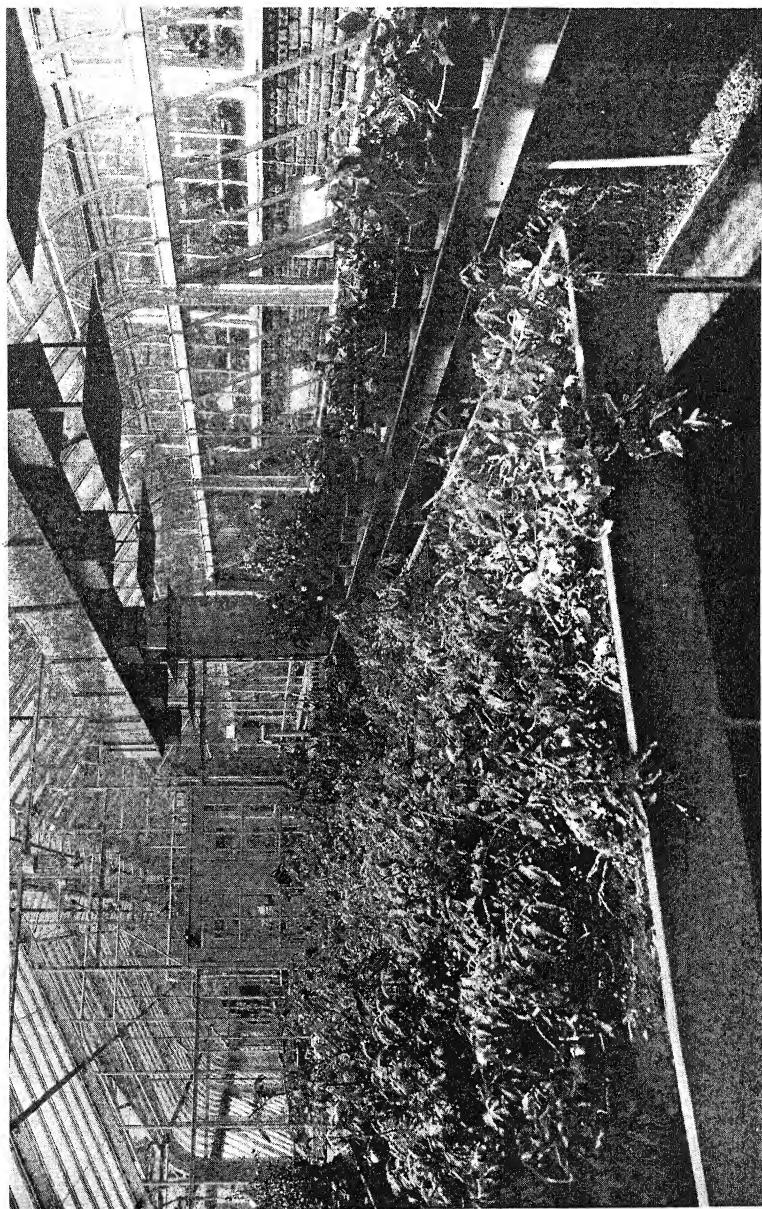


FIGURE 8. GREENHOUSES EQUIPPED FOR HUMIDITY CONTROL.



## BOTANICAL GREETINGS FROM EUROPE

PROFESSOR VERNON H. BLACKMAN

*Imperial College of Science and Technology, London, England*

I feel that it is a very great honor to be invited to come from overseas to take part in this opening ceremony. We in Europe have for some time looked with admiration, not untinged with envy, on the munificent endowments for science which the generous-hearted and far-seeing citizens of this great country have provided. That generosity has been widely flung, for in England also we owe much to such gifts from America as the Carnegie donations to Scotch Universities, and the Rockefeller Foundation's recent gift for medical work in London. America has shown a natural reluctance to associate herself with certain aspects of internationalism, but learning knows no geographical boundaries and in endowing it America is displaying an international spirit of the finest type.

This occasion is surely unique, for nothing in any way comparable with this endowment, fine building and equipment, has hitherto been known in the history of botany. The scientific world has had to wait until now for one, who, like Colonel William Boyce Thompson, not only possesses the material power to found such an institute but combines with it the much rarer knowledge and prescience which enables him to realize the fundamental value of plant research for the advancement of science and the progress of mankind.

It is hardly necessary to stress the fact that nowadays the scientific study of plants touches other sciences and the interests of humanity at many points. It is often, however, overlooked that in this universe plants are the foundation stone in the scheme of living things. As between the two great groups—plants and animals—it is the plants which occupy the premier position. Without the green plant the life of all animals, including that of man, would soon come to an end, for all animals are dependent for their food either directly or indirectly on the green plant. This pivotal position of the green plant in the scheme of nature lies in the fact that it alone possesses the power of manufacturing food materials, since animals merely change one food material into another. The green plant is the great alchemist which alone of living things has mastered the secret of transmuting the sun's energy into food. How this is done is still largely unknown and is one of the pressing problems of plant physiological research, the further elucidation of which is fraught with the greatest consequence to the human race. It is not without the bounds of possibility that, when we have some clearer knowledge of the chain of chemical processes which are involved, we may be able to dispense with the living plant and make our food directly from the sun's energy. I may say that the plant is a

singularly inefficient machine for the purpose, using only about 1 per cent of the energy it receives. It is not altogether a dream to conceive of the scientific man of the future developing an artificial machine of much greater efficiency.

The study of disease in plants is certain to give results of great importance not only to agriculture and horticulture but to human and animal pathology as well. An institute like this which is not responsible for controlling the diseases of any particular area or any particular crop seems peculiarly well-fitted to investigate the more general aspects of plant disease on which progress in some directions depends. Though there are marked differences between the diseases of man and of the lower animals on the one hand and plant diseases on the other, there are also fundamental similarities between the two and knowledge in one field will certainly serve to illuminate the other.

With the realization of the great complexity of scientific problems it is being more and more recognized that difficult biological investigations, such as many of those of human physiology and medicine, are not likely to make much progress unless the attack on the problem is based on the broadest foundation. One may take, as instance, the problem of cancer. The cause of cancer is quite unknown but what is well known is that certain parts of the body cease to act as good citizens working for social harmony in the republic of the body. Instead they throw off their allegiance to the body politic and, starting an independent life, prey upon the other members. Harmony in the relationship of the various parts of the body is replaced by disharmony, leading finally to death. To elucidate the cause of the disharmony is one of the problems of cancer research but the difficulty of the investigation is much enhanced by our ignorance of the nature of the means by which the harmonious development and working of the various parts of the plant body, which is so characteristic of the healthy living being, is achieved. It would clearly be a great step in advance if we could explain this harmony, this coordination between the various parts. What, for example, is the nature of the control which ensures that the two arms of a man grow to the same size, and that the size of the developing leaves is adjusted to the strength of the stem of the plant? The question of the coordination in the living body is one which probably is more easily attacked from the plant side than from the animal. It would seem then that plant research in addition to its contribution to horticulture and agriculture has a definite contribution to make, even to such a very specialized problem as that of cancer in man.

The inter-dependence of the various branches of science is being brought home to us every day and plant research may at any moment illuminate other problems of human physiology and medicine. I may perhaps be excused for referring to another case. Now that insulin

has been discovered, the central research problem in diabetes (for insulin is only a corrective, it does not cure the diseased condition) is the nature of the process by which the sugar circulating in the blood is burnt up in the normal individual but not in the diabetic one. Now this is the problem of the respiration of plants—by what means do they burn up sugar at the ordinary temperature? If the problem were solved or partially solved, it could not fail to help in the elucidation of the problem of diabetes.

If I may, I should also like to express a hope that some of the work of this great and growing Institute will be in the fields which at present have no obvious economic or humanitarian implications. The whole super-structure of applied science is built upon pure science and if the latter is starved, applied science is the first to suffer. One may add a little to the edifice already erected, but without pure science there are no foundations on which to build further.

Some of you may have heard of the reply of Faraday who showed at the Royal Institution of London the experiment which served as the starting point of the dynamo, and on which the whole of the gigantic modern electrical industry is built up. The experiment was the demonstration that you can produce an electric current in a wire by moving a magnet in its neighborhood. After the demonstration of this epoch-making discovery a lady in the audience said at the conclusion of the lecture, "But, Professor Faraday, even if the effect is obtained, what is the use of it?" Faraday then made the memorable reply "Madam, will you tell me the use of a new-born child?" The newly discovered fact of pure science may appear a weakling of little importance or of little economic value at the moment but from it may develop a completely new industry and a new power over nature.

I cannot claim to be an official delegate from Europe, but I have talked with many botanists and they all realize the beneficent activities of such an institution as this where research can be carried out by workers untrammelled by teaching duties and under conditions and with an equipment which in some fields of physiology far surpasses anything that has hitherto been available. The botanists of Europe send their greetings and ask me to express their gratitude to the founder of this Institute for his magnificent generosity and far-sighted outlook. Gratitude has been defined by the cynic as a lively expectation of favours to come. In this sense also the botanists of Europe have a deep feeling of gratitude to Colonel William Boyce Thompson, for under the able management of the Board and with a Director of the scientific eminence of Doctor Crocker, we look forward to research work of the highest quality, work illuminating many dark places of botany, work which this Institute will assuredly achieve.

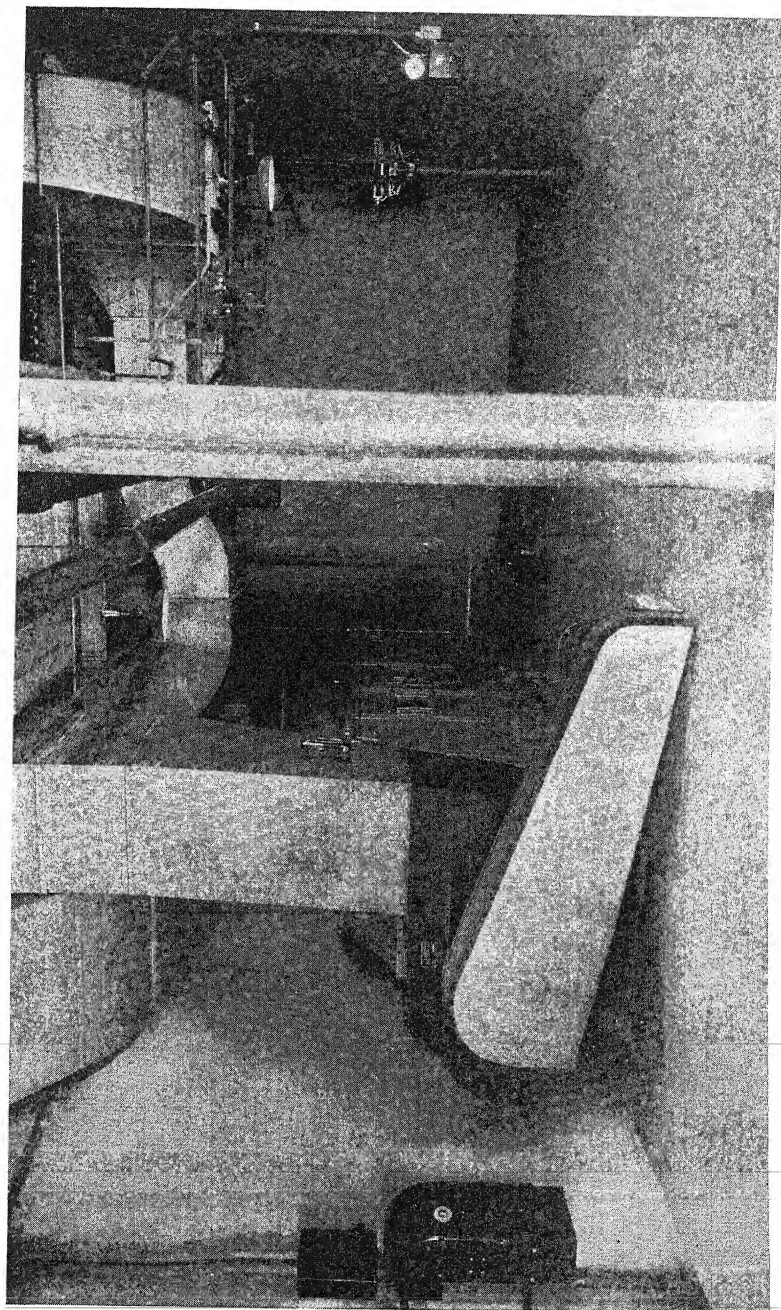


FIGURE 9. APPARATUS FOR REGULATING HUMIDITY IN THE GREENHOUSES

## AMERICA'S NEED FOR PLANT RESEARCH

PROFESSOR LEWIS R. JONES

*Department of Plant Pathology, University of Wisconsin,  
Madison, Wisconsin*

Research is an essential factor in the higher life of man. It always has been—it always must be. Since the “garden of Eden” man has faced the challenge of nature on every hand. From the dawn of human existence when mind-endowed man was “given dominion”—given potential control of nature’s resources proportioned to his understanding of nature’s laws—our intellectual pioneers, in each generation and group of men, have climbed the brinks of human knowledge in eager effort to spy out new fields. Zeal for truth—this instinctive human response to the challenge of the unknown—is the birthright of every child, its maintenance in some degree and kind is an essential to the healthy intellectual life of every man. In earlier days, contributions to human knowledge might come from the layman, working single-handed, a Newton, a Hale, a Franklin, his only endowment native genius and dogged persistence, perhaps the home attic his workshop. But the complexities of modern science demand the laboratory and the coordinated efforts of many men of differing knowledge or skill. What is true of modern business or industry is true of research. The efficient advancement of science requires organization and equipment. Therefore, the maintenance of our scientific self respect, of our racial intellectual integrity, of worthy progress in mastery of our environment, requires that we recognize and encourage research group leadership and that we create research institutions dedicated to serving society collectively where as individuals we can no longer serve ourselves.

Certainly as a plant lover, plant cultivator, plant student, I could not from my study window survey my garden with satisfaction, or in vacation days wander care-free in northern wilds, did I not subconsciously realize that the age-old challenge to ever-deepening, more intimate knowledge of the secrets of surrounding plant life is being sensed and met by the coordinated efforts of many searchers and with the ever-increasing equipments of modern science.

I have aimed thus to express my earnest conviction of this need for research, either personal or by proxy, as a basic element in the intellectual life of man. Any people, any social group which neglects it must undergo mental atrophy. And if this research need results, as I believe, from the inherent human necessity of squarely looking life’s problems in the face, then the place of such an institution as we are today dedicating is fundamental and enduring. For among all of nature’s challenges those which must always be most keenly felt by

man as a living creature are the problems of life itself. The "riddle of the universe" is wrapped in the origin, the operations, the very nature of the phenomena we term life.

"Flower in the crannied wall,  
I pluck you out of the crannies;  
Hold you here,—root and all,  
In my hand, little flower—  
But if I could understand  
What you are, root and all, and all in all,  
I should know what God and man is."

If our intellectual and spiritual needs did not force us to seek to know ever more and more of the laws of plant life, our material needs would so do. Man's success in plant culture is, so far as science can now fortell, the basic measure of the future material progress of the human race.

The world's food problem is at bottom the problem of plant growth.

We can progressively solve it, meeting the increasing needs of an ever-increasing population by increased food production only as we delve deeper into the laws governing plant life processes—nutrition, reproduction, loss by disease.

If, therefore, we accept the inherent needs of man's intellectual life as the fundamental justification of permanently endowed research, we may turn to his physical needs as arguing for a liberal share of any funds dedicated to the social betterment of the race.

Rarely, I believe, has there been clearer promise of reward in the research field than is the case today with certain plant problems.

If specific illustrations are desired one may turn most wisely to his own professional field. Let me invite consideration therefore of the opportunity and need for research upon the problems dealing with plant diseases—their nature, cause and control.

### *The challenge of the mosaic diseases*

The importance of germs as causal agents in disease holds for plants as for animals. The germ theory of disease, based on the modern conception of parasitism, was, indeed, first established with plants. But although for two generations we have been perfecting our knowledge of the parasitic fungi and bacteria which cause rusting or blighting of crops, we are today challenged by a disease complex more baffling and possibly more serious than any ever faced before. Each year there is an insidious increase in the prevalence of the so-called "mosaic" maladies upon our plants, yellowing, mottling and dwarfing foliage and fruit with disastrous results. First one crop plant and then another has fallen subject to it,—peach and cane fruits, tobacco and

cucumber, potato and tomato, lettuce and spinach, corn and sugar beet, aster and dahlia. Each year the list lengthens among our food plants and flowers;—but quite as significant is the fact that many wild plants and weeds are also included. The especially portentous thing is that this type of disease is subtly infectious like the comparable “virus” diseases of animals,—the hoof and mouth disease, rabies and small pox. No microscope has revealed a causal “germ,” but whatever the nature of the “virus,” it is readily carried by sap-sucking insects from diseased to healthy plants. Even more startling is the frequency with which the “virus” has proved inoculable from one type of plant to another. Thus it may be transmitted from cultivated plant to weed and after over-wintering in the fence-row-perennial, again return the next season to ruin the adjacent field crop. For some years the increasing loss with potato and other food plants from these virus maladies has convinced pathologists of their seriousness. This was, however, recently brought home in a most striking way to commercial interests when the sugar cane fields were invaded by it—first in Hawaii and the Orient, then in Louisiana, now in Cuba. The possible significance in cost of living resulting from a world-wide crippling of the sugar industry is startling. Were we in the United States not sufficiently alarmed by such a suggestion we may add the further possibility that this cane “virus” may be transferable to corn. What more alarming tax could be imposed upon American food products than the affliction of our basic crop—Indian corn—with a serious toll-taking disease?

What is to be done about it? One thing, and one thing only,—research, and more research! Find out what is the nature of this “virus,” the cause of this insidious new plague creeping over our chief crop plants. The evident and temporarily expedient things will, of course, be cared for by our present scientific organizations, either as supported by public funds, or with emergency financial help from the industries threatened. But the long-time basic studies essential to the ultimate solution of this and similar problems, need the encouraging stability and resources of research foundations permanently endowed for such purposes. I am glad to note not only that this has been assured by this Institute but that important additions to our knowledge of certain “virus” diseases are already coming from these new laboratories.

I might cite other things;—concerning the need of researches upon the fundamental nature of parasitism, for which we look for leadership and advice to our English guest of honor; upon the relation of environment, temperature, moisture, light, atmospheric composition to the plant functions—whether in health or disease—for which our Scientific Director has already equipped this Institution in a way heretofore undreamed of; or upon the possibilities, through plant breeding or



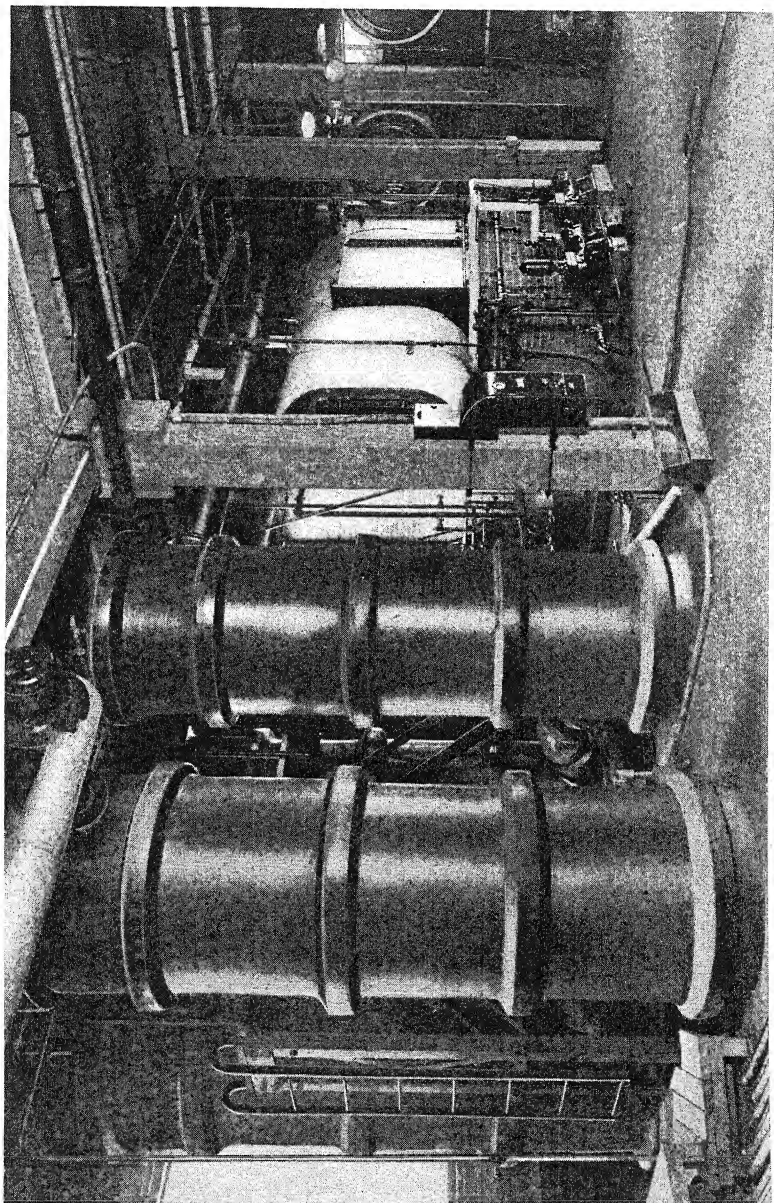


FIGURE 10. FLUE-GAS SCRUBBERS FOR WASHING CARBON DIOXIDE FOR THE GREENHOUSES



selection, of progressively perfecting sturdier strains or types of our ornamental or crop plants—which through resistance to disease may solve, let us hope for all time, the problems of control of certain plant plagues which now exact their profit—taking tax from garden orchard and field. As earlier said, there never has been a time in human history more opportune for plant researches with assurance of worthwhile progress.

Some may still question whether the several State and National agricultural institutions supported by public funds are not adequate for research upon these and related problems dealing with plant disease control and other lines of applied botany. There can be no doubt that the responsibility should remain with them to meet local and temporal needs. But these very things must in the future as in the past inevitably distract and drain a vital part of their resources and energies. Tax-supported institutions, and the men located at them, cannot as a rule follow at will the fundamental aspects of research problems even where these develop out of the applied aspects. Far be it from me to say that they cannot and should not do this in some measure,—so far, indeed, as is practicable and compatible with their immediately pressing responsibilities. I should much regret it if anyone interpreted these statements as indicating any lack of emphasis upon either the rights or duties of workers in State or National institutions concerning the fundamentals of science underlying their problems.

Emphatically would I affirm, that it is out of my very faith in this right, born out of long experience in such State work, that I define and defend this need for help through endowment. Several years ago this need for an amply endowed research institution to supplement those now existing was clearly voiced by the American Phytopathological Society. It is noteworthy both that the Institute we now dedicate corresponds in essentials with the conception of this national society. It is further to be noted that it is the clearly-defined wish of its founder that this Institute shall supplement and not supplant any other existing research agencies. From its open doors stimulating and strengthening influences are radiating. From these, at last, comes assurance that whatever may be the fundamental lead opened by anyone in such other institution, here may be opportunity and facilities for its ultimate development.

If we recognize this need in plant pathology even more clearly does it exist in these other lines of plant science, like physiology, where the applications are not so evident or so directly sought. Opportunity has heretofore existed along these lines in many of our universities and in connection with a few botanical gardens and similar scientific institutions. But in none of these have we had either the assurance of substantial financial support for new lines of research or adequately equipped laboratories and greenhouses in association suited to experi-

mental work with plants under controllable environment. Perhaps the explanation is in part that botany has not heretofore had a benefactor who combined in like degree, wisdom of decision with ability to realize upon his ideals. While gratefully recognizing this we should, however, further recall that never before have the contributing sciences of physical and chemical engineering been ready to equip houses in the ways which we have today seen. For a generation plant experimentalists have been wishing to learn more exactly the relation of environment, temperature, light, atmospheric composition, to plant nutrition, growth and behavior in health and in disease. But never before has it been possible to devise and install air and light conditioning apparatus at all comparable to this. In this connection it is especially pleasing to note that it was the pioneer work of Doctor Charles F. Hottes of the University of Illinois, the long time teacher of our director—which paved the way for these present developments upon the large scale.

And finally, since we have spoken in a congratulatory vein upon what has been already perfected in material equipment and of what is generously assured in the way of financial support, I wish to voice a more conservative conclusion. We may wisely remind ourselves that nature is most jealous of that which is of greatest worth among her secrets. It is only to the searchers of enduring patience and most exacting skill that she will yield important new truths. No one should expect epochal advances in easy succession from the staff of even so well-equipped an institution as this. Let us not desire the spectacular, but the normal. Quiet, persistent progress, month by month, year by year, generation by generation; it is thus that most is accomplished in research as in all other lines of useful human endeavor.

Let us not be deceived as to the price of modern research work, whether measured in terms of time, man-power or money. As we before reminded ourselves, the time is past when the isolated man working apart from others may hope to proceed far with most types of plant research. Individual initiative is of course essential and counts as never before; but the idea being formulated, the question defined, its fundamental development may be conditioned upon the correlated endeavors of experts in several fields, often involving biochemistry and biophysics quite as much as biology, as narrowly defined. This means either that the activities of even this institution must year by year be narrowed in scope or else we must base our hope on the faith that the results secured shall so commend themselves that continually increasing endowment shall adequately support the future developments so splendidly inaugurated in the Boyce Thompson Institute for Plant Research, which today we dedicate.

After the preceding addresses the following impromptu remarks were made by prominent guests.

DOCTOR RAYMOND F. BACON—*Consulting Chemist and Member of Board of the Institute.*

Through the speeches which have just been made, and from your inspection of the building this morning, you all perhaps realize that The Boyce Thompson Institute for Plant Research is quite a wonderful institution, and doubtless the thought occurs to you as to what manner of man it is who had the vision to create such an institution.

A few years ago Senator Beveridge wrote a most delightful series of articles dealing with the great men of the world of the passing generation, and particularly the Marquis Ito, Cecil Rhodes and Count Witte, outlining the characteristics which had made these men great nation builders. He pointed out that all of these, as well as almost all other great men, had certain predominant traits.

First: These men were all dreamers. In those quiet hours when one communes with himself, the vision had come to them of what they would do in the world, and this vision had been elaborated until the eye of the imagination saw a completed picture of something which had not before existed. A picture of something which would make the conditions of the world better and happier for posterity.

A second characteristic which made these men great was singleness of purpose. Once the dream picture was painted, all of a tireless energy went to make the dream a reality.

A third trait was a certain directness of action; the ability to seize the significant facts and go in a straight line from fact to fact so as to cast into steel and cement the thought of the mind.

It was many years ago (before that magnificent house on the hill was built), that I first heard of the Boyce Thompson Institute. Late one night Colonel Thompson and I were walking in the grounds. We were speculating on the nature of many of the fundamental things of life, and he was allowing his very active mind to express itself aloud. He finally said, "When I have made enough money to do it, I am going to build an institute to study some of these fundamental things. I should like to get at the real bottom of the phenomena of life processes, and I think a good place to study them would be in the realm of plants. By doing that perhaps I can contribute something real to the future of mankind."

Colonel Thompson was born in Montana, and spent his early life on the plains of that great state, and perhaps this contact with a country where nature does things on an enormous scale gave him largeness of vision, for all that he has done has been done in a big way. And on the other hand, he has an almost superhuman patience in

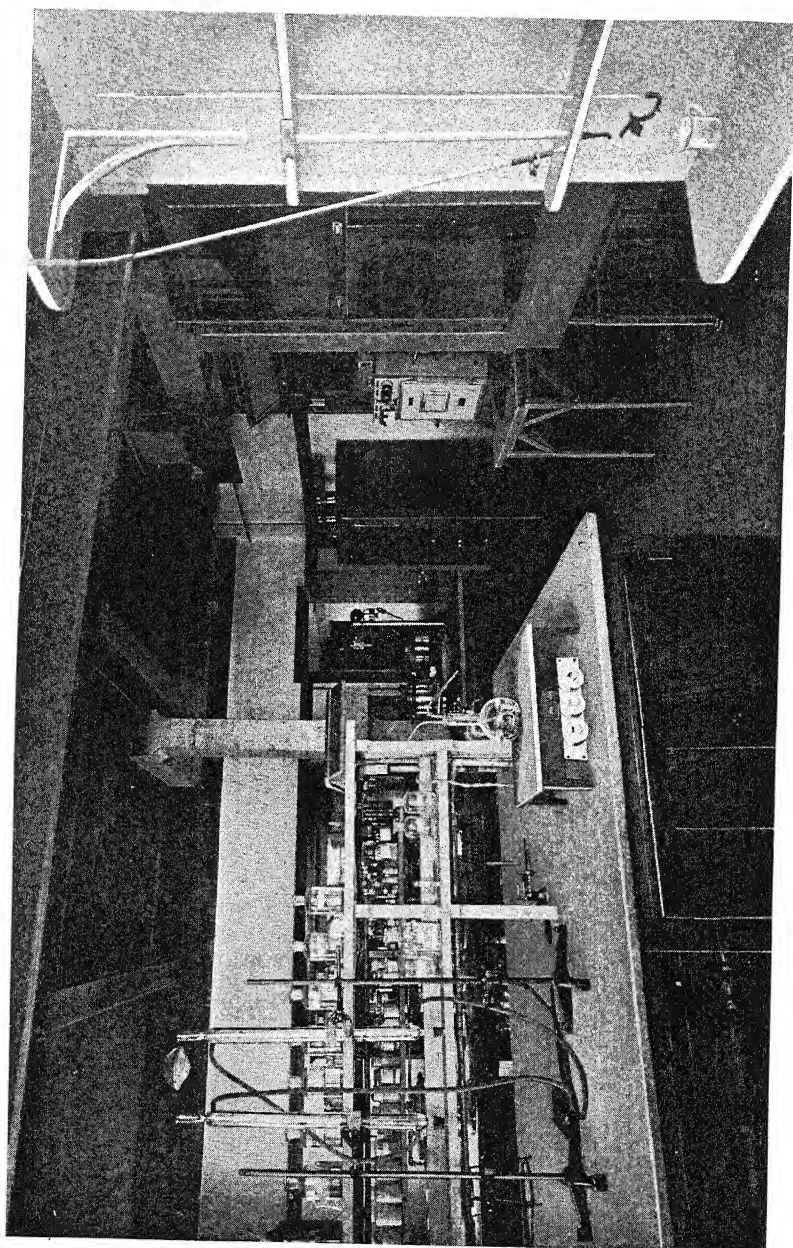


FIGURE 11. A BIOCHEMICAL LABORATORY

working out the details which mold the big conception into a perfect realization.

Many of you may have admired the wonderful landscape effects, or the beautiful gardens on his estate, and you may have thought that sufficient money can buy such a place without much thought or effort on the part of its owner. I tell you that the Colonel knows every flower, every shrub and every tree on that place, and he has himself put every one of them in its proper position to make the picture that his imagination had created. And so it has been with the Boyce Thompson Institute. He had dreamed of it in a big way, and then, with intense singleness of purpose, he bent everything toward mastering the necessary details to make the perfect realization of his dreams.

In any place as beautiful as that estate across the road, or in any institution as wonderfully equipped as the one you have inspected today, it is not a mere question of money to buy materials or other men's services. It means an active directing brain behind it all, and the brain in this case has been that of the Founder of the Institution.

In building this Institute the Colonel has shown another of those traits of the great men of history in his ability to seize on the significant facts, and make proper judgment from them. He has a real passion for getting at the true facts of a situation. He talks to man after man to be sure that the facts given to him by experts in their respective lines are correct. When he has finally sized up the situation, when he is sure that he is right, then he goes ahead full steam and there is no stopping him. And that is the way Boyce Thompson Institute has been built.

Perhaps from this picture of the terrific driving energy of an efficient brain, you may think of the type of man that we admire at a distance, and sometimes speak of as the cold calculating machine. Colonel Thompson is not that type of man. Those of you who know him know his kindly and wonderfully magnetic personality. He draws friends to him. He is the kind of a friend you love to be with.

This Institute which he has dreamed and made a reality will discover many new scientific facts and develop many new ideas. Facts and ideas are living things which never die, but go on down through the ages, and others build on them and because this man has lived and has been the kind of a man he is, our children and their children's children will have a happier and an easier life. Or may I express it this way; theirs will be an era of more gracious living because this man, whom we are proud to call friend, has had a wonderful vision far into the future, and has been a great enough man to make his vision a reality.

COLONEL RAYMOND ROBINS—*Social Economist, Chicago, Illinois.*

Dr. Coulter, Dr. Crocker, honored guests and ladies and gentlemen, friends of the Institute, I shall begin my extemporaneous remarks similarly to those you have just heard.

Mr. Chairman and Director and friends: I count it a privilege to be able to read to you these brief words:

"The White House, Washington, September 23, 1924.

"My dear Colonel Thompson: This is to express my appreciation for the pioneer work you are doing through the Boyce Thompson Institute for Plant Research, Inc. The scientific mastering of the problems of plant life will strengthen that base of all material welfare—agriculture. This is the new pioneering in the American Advance.

"Yours very truly,

CALVIN COOLIDGE."

I want to say what I say on the basis of that text, taking first the word *pioneer*. It is the typical word, the spirit in the life of this America. All that is worth-while in the story of our national life has come from the vision and the dreams of the pioneer made real.

I am one of those fortunate persons who have stood with William Boyce Thompson there by the sands, beside the bank of Alder Gulch, where he was a lad, and have seen that world where he first caught his vision,—have sensed the fact of his pioneer mind. And now that the frontiers are closed, now that there is no longer a frontier on this continent, he is pioneering in other ranges of the mind. You can reach the limit of the material universe but you will never reach the limit of the scientific ranges of the human mind and spirit. And it is in that sense that the word pioneer seems to me especially fitted to this occasion.

If I may say so, Dr. Crocker, to you and to your able assistants, there is something in that thought that no matter how closed the old frontier may be, the great frontier of science is as wide and as open as the human mind.

The other thought, "the scientific mastery of the problems of plant life." What a splendid phrase that rather Silent Bit of New England Granite puts into that brief word. I saw him yesterday on other matters in the midst of a Cabinet meeting and great affairs, and he said, "You must take my greeting to Col. Thompson," and then he wrote these words. He said, "I wish you would also say to Col. Thompson that I wish him well in the matter of his health and strength and I wish well to all those engaged in the great task that he has made possible at the Boyce Thompson Institute for Plant Research. For we are now facing," said the President, "an hour in relation to the future resources of the world, in our own country and in all the world, when the question of agricultural research will be the fundamental material basis of everything worth the while."

Unlock the mysteries in the chambers of life! That seems to me to fit this afternoon. I am thinking of that plant (pointing toward the Boyce Thompson Institute) over there and its tremendous resources. Then I am thinking of what one of the greatest and most noted painters of the world once said. When someone asked him, "How is it possible to get a picture like that?" He said, "I mix my colors with brains." The plant is not worth very much except as it is mixed with brains, unless there is the kind of human conception such as Tyndall gave when he dared to risk blindness that he might go forward with his tests. There is a certain moral equivalent for the old qualities in war in this magnificent advance of science into the realm of mystery, unlocking these mysterious chambers of life.

I am thinking that as a matter of fact this afternoon, having been the pioneer on the frontier, William Boyce Thompson is engaged in being a Columbus in an entirely new realm and new adventure. Never before in the story of the human race has there been so adequate an equipment, so splendidly officered with intelligence for the solution of some of the major problems of the life of the world.

I think it is to you, sir (turning towards Dr. Coulter) that I am indebted for the thought that all the great progressive development and wonder of the world's life is due to the impulse to do something never yet done. That "creative evolution," if I dare use that word, lies behind that old desire to do something never yet done. And I am thinking here this afternoon that those of us charged with any responsibility in this great task are traitors betraying the dreams and the adventure and the daring of a million years if we do not make good on what is here. And those of us who do make good, wherever you are and in whatever line the task, with however limited equipment—those of you who do make good are fulfilling once again the dream and the daring of a million years.

I care for that larger heritage for the generations yet unborn, and in all the larger ways it rests back on the fulfillment of our task in our generation. And I dare conclude what I would here say in the thought of One—the Supreme Pioneer of history. Wishing to be reverent, yet I, none the less after very intimate association with William Boyce Thompson, through long and trying years in the storm and terror of the Russian Revolution, where in all that complex he found the line of truth and told it to his government,—although it was too much for the government to find it and seven years had to roll away before even Great Britain could find it—but they will find it in the end—I think of Colonel Thompson and then I think of Him who said, "I come that they might have life, that they might have it more abundantly."

That seems to me to be the heritage of the Boyce Thompson Institute for Plant Research—the more abundant life for all mankind.



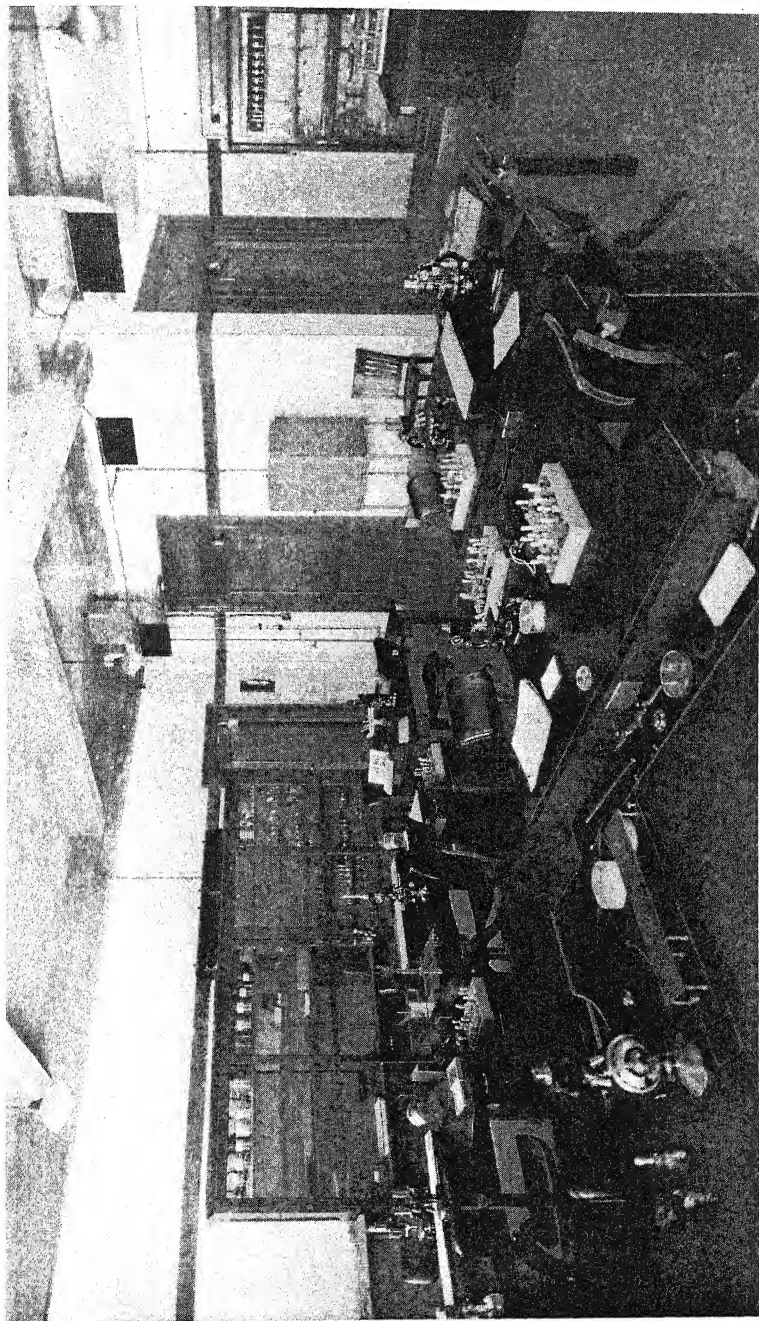


FIGURE 12. A MICROCHEMISTRY LABORATORY

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DR. ELMER DARWIN BALL—*Entomologist and Director of Research, United States Department of Agriculture.*

Mr. Chairman and fellow scientists, I would like to bring to this meeting today the most sincere greeting and words of encouragement from the Secretary of Agriculture and from the great body of scientific workers of that Department. In the history of this country there has never been an institution established that we consider more vital and more fundamental to scientific progress or that we welcome more heartily than this undertaking.

As I listened to the other speakers cover so adequately the possibilities and opportunities of this Institute I could not help but go back in my mind to the early seventies when it was almost universal in the farming country in which I lived to decry wealth and to denounce those who accumulated it, and yet that very region owed its development to the organized wealth that built its railroads and founded its business enterprises.

It has for a generation, as you well know, been politically popular to decry wealth, especially organized wealth in the form of trusts, but when we undertook to carry on a war we found them not only useful but necessary and started out to form new ones. Business long ago found out that organization of capital was necessary to the carrying out of large and vital undertakings and our government found that the same thing was necessary when it undertook the war.

As wealth accumulated in this country more and more of it was turned towards the endowment of education and the result has been to materially increase the opportunity for national development. Later a new avenue of opportunity for service was found in the endowment of research and in such endowment, as you would naturally expect, the first provision was for the unfortunate and so they endowed medical research. We are today celebrating, as has already been called to your attention, an entirely new epoch in the history of the service of wealth to national development. It was left for Mr. Thompson to create the first large scale, large visioned endowment of research into one of the fundamental problems of agriculture, a problem of such importance that every contribution towards its solution is a direct contribution towards the development and continued maintenance of a growing nation.

In closing I would like to make it clear to our visitors from across the waters that in this program we have no imperialistic ambition. We are, however, beginning to realize that an extraordinary effort must be made to increase our food production to keep pace with our expanding population and that ultimate success in this undertaking requires the prosecution of fundamental research in every field related to the problem. Our ambition is to grow and develop into one of the strongest and most powerful nations of the earth, not for the purpose of terroriz-

ing other nations, but to assist in stabilizing the leadership of the world along scientific and economic lines that will contribute to permanent peace and a developing civilization.

DR. ROBERT A. HARPER—*Professor of Botany, Columbia University, New York City.*

Mr. Chairman, ladies and gentlemen: It is certainly a great pleasure and privilege to be permitted, on such an occasion as this to speak in behalf of the Universities of this country, at least in so far as they are represented by a near neighbor of this Institution, Columbia University, and to try to express their feeling of warm sympathy and great pride and strong encouragement in the work that they are doing, when they see and realize, as we are today realizing, what this new movement for the advancement of knowledge really means.

Research in plant science has been in the past a sort of by-product of other activities of the human kind. The first scientific knowledge of plants came, perhaps, as a by-product of the activities of the medical profession and the herbalists who found plants useful in curing the ailments of suffering humanity. The universities, we frequently say now, may regard research as one of their by-products. Universities have a great function in handing on from one generation to another the accumulated wealth of knowledge and scientific practice that each new era builds up. Many believe that the permanence and the progress of civilization depend on nothing more than the successful handing on and wider dissemination of this increasing wealth of knowledge. To transmit to each new generation of young men and young women the accumulated wisdom and science of the past is certainly a worthy function, on which the universities may always rely as a justification for their existence and further growth.

But the universities were the first, I think, to realize that the accumulation of new knowledge by special effort, specialization in the advancement of learning, is the supreme function of all those who are really interested in the intellectual as well as the economic life of men, and they have been moving toward making research something more than a by-product.

On behalf of the universities I am sure it is further appropriate that I voice the feeling of responsibility which we, as scientific men, must feel in participating in the dedication of an institution like this, which aims especially to devote itself to the study of the more fundamental problems of plant life and growth. It is no light matter, not only to plan for the attack on old problems which have so far resisted the efforts of the scientists, but also to attempt to discover and formulate those problems and lines of advance which are as yet only vaguely realized. I am sure we all agree as to the importance of an institution of scope and organization adequate for this sort of work. State-supported

bureaus and experiment stations can get funds for the investigation of problems that seem practical and more or less readily soluble by the application of fundamental principles already known, but it is perhaps our greatest present need to reach out into the larger fields where lie the problems which have been pronounced seemingly insoluble, and perhaps even ridiculous, and to formulate these problems so that they can be attacked by experimental methods. In the formulation of plans for research, the real importance and significance of the problems to be attacked should, perhaps, be more considered than the seeming probability of their being readily soluble.

This may sound unpractical, if not chimerical, but I am sure we all feel that, with such equipment as we have been inspecting here today, and with such a program as has been outlined by the administrative officers of this new Institute, fundamental research is becoming something very tangible.

In closing, may I voice the wish and hope of the universities that this is only the first of a great series of sister institutions devoted in aim to that which the universities also regard as their highest function—advancement in knowledge.

DR. LEWIS PERRY—*Principal, Phillips Exeter Academy, Exeter, New Hampshire.*

Mr. Chairman, ladies and gentlemen, it is a very tantalizing honor to be called on at this particular time in the afternoon to give a two minute extemporaneous speech—yes, sincerely, an extemporaneous speech—in regard to Colonel Thompson. But perhaps it is the treatment which you always give to the schoolmaster when he is away from his school. Only recently I had another experience that illustrated much the same thing. I had given a commencement speech and the head of the school, who was a lady, came forward to my great surprise and handed me what she called an honorarium—this is a true story—and I refused the honorarium. It was a great pleasure to be allowed to speak at all and I appreciated that honor, but I would not take the money. She said "You must take the money." I said, "No, really I won't take it. I have enjoyed myself and I won't take the money; use it for some fund." And a great light broke over her face and she said, "That is a very good idea. I will put it into a fund to secure better speakers."

I think there are only two distinctions which I have as a speaker this afternoon: in the first place, I am the first one who has spoken who is not a scientist. In the next place, I am the head of the school where Colonel Thompson graduated. Daniel Webster and Colonel Thompson were among my old boys, and if among my old boys you ask of which one I am proudest today I do not think I could tell. I put them both on a par.

A great American has said that there are three lines of development which may come to a man, and I think Colonel Thompson has lived out all of them. In regard to Colonel Thompson's birth; I want it distinctly understood that he was not a pioneer at first. He went to Exeter before he became a pioneer. The education which he had and which he took and which he profited by—that was the first period.

The next great period of a man's life, according to this American, is the period of acquisition. Now, I am not capable of speaking about that, but from having looked at the minerals this morning and the greenhouses and this great Institute and taking rather a scientific attitude towards it, I imagine that there must have been in Colonel Thompson's life a period of acquisition.

Third, a period of service where the education is utilized and where the money which has been acquired is used for the service of mankind. In all these three things Colonel Thompson has been preeminent and his old school, I can assure you, while it is very proud of this Institute, is prouder still and fonder still of the man himself.

DR. ROSCOE W. THATCHER—*Director of New York Agricultural Experiment Stations, Geneva, New York.*

Mr. Chairman and friends, in speaking on behalf of the state-supported institution for research in welcoming to our field this philanthropically-supported or, perhaps I should say, more adequately-supported Institute, I am reminded of the old wise saying that where a man's treasure is there will his heart be also. Some of you may be aware of the fact that the Republican State Convention is in session at Rochester this afternoon and that the Democratic State Convention will be in session at Syracuse tomorrow. I suppose you thought perhaps that I would be there. This more or less politically fictitious method, whereby the American people are being wisely (?) guided into the selection of those who shall rule our destiny for the next two years, is in progress; but my heart is not there, even though I am afraid that my treasure is there, at least the keys to the treasure are there.

There are one or two things that those of us who represent state-supported institutions for research may say to you, which we may say with sincerity and honesty and perhaps in a way that no one else could say with as much feeling.

Four weeks ago yesterday I stood on the top of the continental divide in central Montana. I looked out over an area of forty to one hundred miles in radius and I saw what nature had done in the way of piling up the backbone of this continent. I did not know until a few moments ago that I was looking up Alder Gulch which has been referred to as one of the places where Col. Thompson secured his inspiration. But I think I can understand something that perhaps has not come to any one of you by reason of that experience.

I saw four forest fires in that area. I had talked with the forest supervisor and he had regretted that the public policy was such as would prevent making it possible for him to put trails through that wonderful forest so that he could reach those fires quickly and efficiently. When Dr. Coulter this afternoon alluded to the possibility of our exploring an unknown country and said that in his estimation it perhaps would not be quite so fine a spirit if we built trails directly to a known gold mine or to a great source of power as it would if we had built those trails in order that we might find and worship nature's handiwork, I thought perhaps after all that Col. Thompson has seen something in those Montana forests which led him to think that it would be wise to make trails in such a way that they might contribute to some very definite purpose. I have every sympathy with the attitude expressed by Dr. Coulter when he says that we may have a higher motive than the purely practical in research. But if, on the other hand we build a trail toward a certain well-defined goal we will make it in a certain definite way, and I think that is what Col. Thompson, through his wisdom and that of his associates, is doing here; and the way that he is doing it, which is not possible for the state-supported institutions, has appealed to me all day.

Research may be defined in all sorts of ways; but to my mind research is the application of the experimental method of study to the laws of nature. The experimental method, as I understand it, is the study of natural phenomena or data in such a way that one can determine a cause and effect relationship. One is not simply accumulating facts but is accumulating them in such a way that he can be sure to determine a cause and effect relationship and so argue from the observed effects to the underlying cause.

In research institutions we are asked to do just that. But the pressure on state-supported institutions is for some immediately practical result. Our state, and when I say our state I mean the American commonwealth, is not so very patient with the attack upon a problem which is not specifically directed towards an obvious end and it is not sympathetic to an attack which does not lead to so-called practical results. In the field of plant science the experimental method requires the controlling of the environmental influences in such a way that one can argue correctly from the observed effects to the preceding cause. We have been able to do this to a degree in some institutions, and I think I may proudly say that the one I represent is one of those which has been characterized as being able to do some of these things in the field of science as applied to agriculture. We have been able sometimes to control the temperature and perhaps other environmental factors and so to get at certain facts. But in this great field of natural botanical study it is not possible for those who are working in state-supported institutions to do what is being done here at the Boyce

Thompson Institute for Plant Research, to control simultaneously all the factors whereby nature works in her magnificent laboratory of the plant cell.

I am not very hopeful, after twenty years experience in dealing with state legislatures, of being able to put over to state legislatures the idea that successful research in these fields of plant science needs the kind of equipment and support which we have seen exemplified here. Hence, it is with all the more real pleasure that I come here to welcome to the corps of research workers and the research equipment of the country an Institute which is founded on such a broad vision that it will provide as well as can possibly be done for the controlling of all environmental factors in studies of plant growth. In the past, the influence of certain factors, especially those of the kind and amount of light received by the plants, has been overlooked, and sometimes wrong conclusions were drawn because we did not attribute the observed effect to its proper cause.

Whatever may have been the stimulating influence in Col. Thompson's mind, whether it was that view of Alder Gulch which I saw from the continental divide, or whether it has come from his business experiences, or whatever the stimulus was, it is something upon which we all are to be very greatly congratulated that we have now this wonderful opportunity, within the borders of our own state, to come nearer at least to the ideal facilities for research.

DR. JACOB G. LIPMAN—*Director of New Jersey Agricultural Experiment Station, New Brunswick, New Jersey.*

Mr Chairman, Colonel Thompson, ladies and gentlemen, in this land and in other lands there are men who are devoting themselves to the study of soils. Some of them are geologists. Beginning with the study of rock formations they attempt to inform themselves and others of how rocks are gradually changed into soils. There are others who are chemists and they make an inventory of the plant food resources of the soil and report how many pounds of phosphorous or potassium, or calcium or other constituent elements of plants are present in these soils. They are also studying these soils as laboratories wherein transformations go on that make possible the absorption of nutrients by plants so that the latter may become available as animal or human food. There are others, calling themselves physicists, who are engaged in the investigation of the movement of moisture and air in soils and of temperature relations and other physical effects that have to do with plant growth. And there are still others calling themselves soil microbiologists who are engaged in the study of bacteria, protozoa, fungi and algae and small animals, insects and so forth, and their relation to plant production. In other words, there is a large field of

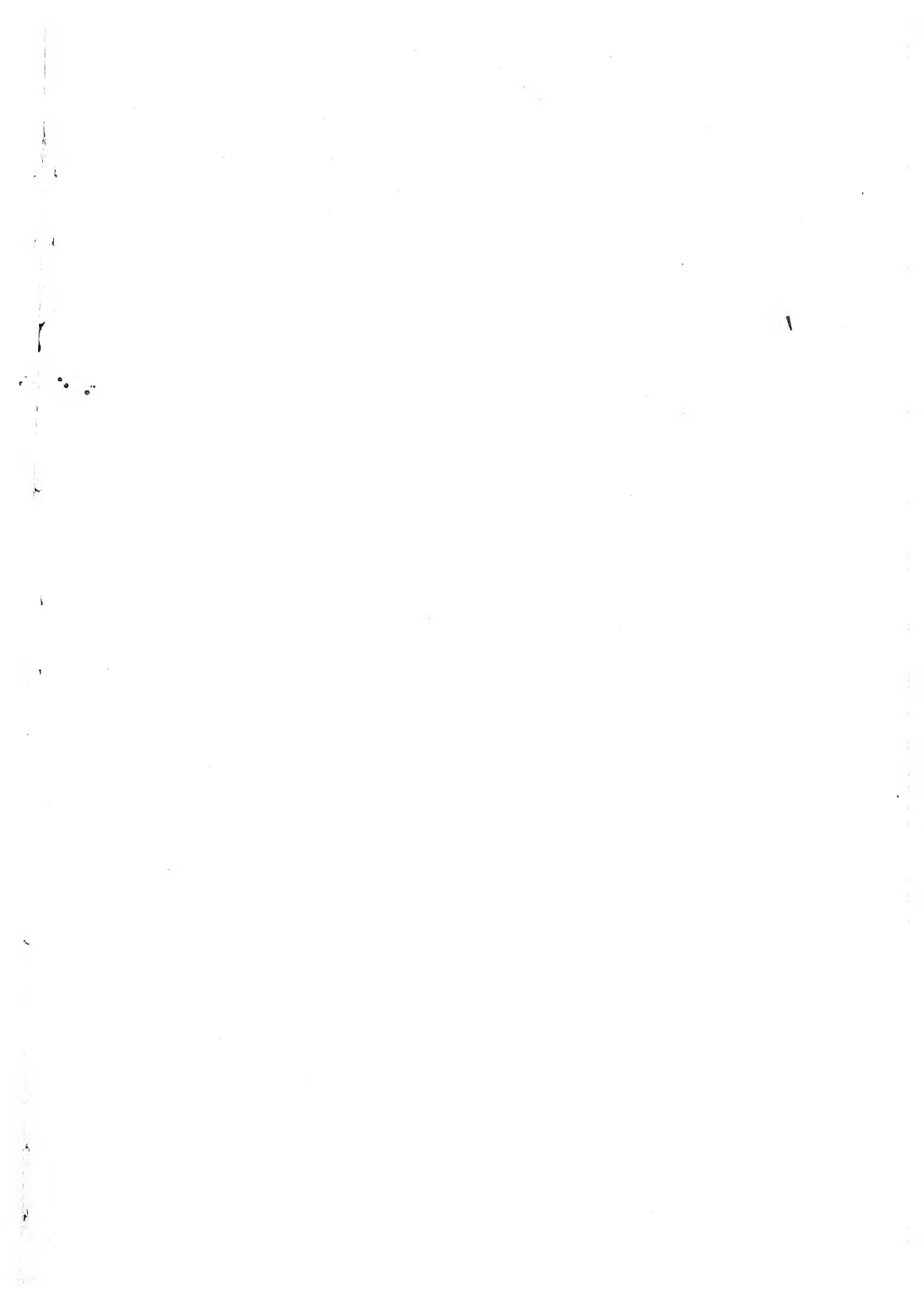
soil science which has a very intimate connection with this field of plant science that we have been speaking about this afternoon.

In 1908 a group of soil investigators met in Budapest, Hungary and organized what was known as the first congress of soil science.

Another international conference was held in Stockholm two or three years later. A third was to be held in St. Petersburg, as it was called in those days—I think it has changed its name two or three times since then—but on account of the war it was not held. And finally, two years ago, workers in the field of soil science met again in Prague, Czechoslovakia, and organized the study of soils in a more systematic way. They met again in Rome early this summer. They decided to organize an international society of soil science, and have done me the honor to elect me president of this society. They have voted that the next international congress is to be held in Washington, D. C., in 1927. I think it would be quite proper for me to express greetings in behalf of the many hundreds of soil investigators in many lands to this Institution and to all of you who are interested in its welfare and its future.

We all realize that the soil is the foundation on which plants must grow and that whatever resources of plant food there may be in the soil, the environmental factors would naturally affect the yield of animal and human food. We recognize that there is such a thing as an efficient plant, efficient in utilizing the sun's energy, efficient in resisting the invasion of parasites, or efficient in its ability to grow at lower temperatures. All these factors are intimately related among themselves, as they are related to specific soil fertility factors. To those of us interested in the field of soil science—and the dividing line between soil science and plant science if it exists at all is quite indistinct—this is a red-letter day. We rejoice in the fact that this Institution will make more ample the resources of plant and soil research.

I wish to convey again in behalf of those for whom I am taking the liberty to speak their greeting, congratulations and best wishes. I wish, likewise, to express the hope that there will be the fullest cooperation between those engaged in the study of soils and those engaged in the study of plants in order that we may make the largest and most helpful contribution to the progress of human kind.







## HASTENING THE SPROUTING OF DORMANT POTATO TUBERS

F. E. DENNY

(Received for publication December 4, 1925)<sup>1</sup>

### INTRODUCTION

Freshly harvested potato tubers, if replanted under conditions favorable for growth, do not sprout at once, but require a period of after-ripening. The depth of dormancy varies with the variety but is generally two months or more.

This delay in sprouting is a serious disadvantage to potato-growers. Thus, Bliss Triumph seed tubers grown in northern states, such as Maine or Nebraska, do not go through their rest period in time for planting in Bermuda, Florida, Cuba, or other southern localities in which an early crop is produced for the winter market. It has been necessary in such cases to obtain seed potatoes from Long Island or regions farther south, where the first crop of potatoes matures early enough to give a period of after-ripening sufficiently long to insure prompt germination in the autumn of that year. But such potatoes have been found generally to be subject to infection by degeneration diseases which either greatly reduce the yield of crop or cause practically a complete failure. It would be of great value to potato-growers if a method of shortening the rest period could be devised so that disease-free northern-grown seed potatoes could be taken at once after digging to southern localities and used as seed for the early winter crop.

Again, in many southern states a second crop of potatoes is grown in the same year on the same piece of land; but the sprouts are slow in starting and the stand obtained is irregular, in consequence in large measure of the dormancy of a certain proportion of the buds planted. Hence, the yield per acre is decreased materially below the normal productive capacity of the land.

Experiments for the purpose of finding a method of chemical treatment that would shorten this rest period and permit early sprouting were undertaken. While the work at present is incomplete, certain of the treatments have given hopeful results, and this paper is a preliminary report of the progress made.

### METHODS AND VARIETIES

In some experiments the tubers, both whole and cut into suitable sizes for planting, were soaked in aqueous solutions of different chemicals of

<sup>1</sup> Published, at the expense of the Boyce Thompson Institute for Plant Research, out of the order determined by the date of receipt of the manuscript.

gradually increasing concentrations for varying lengths of time, and in others they were exposed in closed containers to gases or vapors from numerous volatile compounds. After treatment, the potatoes were planted in sawdust or in soil, in the dark or in a greenhouse during cold weather, or in the Institute gardens during the summer months.

The varieties used were: in December, 1924, Irish Cobbler, Rural New Yorker, and Green Mountain from Maryland and New Jersey; in January, 1925, McCormick from Washington, D. C., and New Jersey, Green Mountain from New Jersey; in February, 1925, Bliss Triumph from Bermuda; in April, 1925, Spaulding's Rose from Florida. Since the potatoes mentioned above were not deeply dormant, the results of the experiments with them were used merely to indicate the treatments that gave the most promise of success. The conclusions reported in this paper are based for the most part on the experiments with potatoes from the following sources: Irish Cobbler from Long Island, 1925 crop; Irish Cobbler, Bliss Triumph, and Early Ohio, 1925 crop, grown in Institute gardens, treated and replanted within one month from the time of digging.

Early forcing of sprouts was obtained with all these varieties by one or more of the chemicals used except in the case of Early Ohio. In the only experimental series with tubers of this variety, none of the treatments used was successful.

## RESULTS

### General Statement

Two hundred twenty-four different chemicals were tested and about 3,000 separate experimental lots were used in selecting suitable concentrations and periods of treatment. In this report only those that gave the best promise of success will be discussed, but it is hoped that in a later publication a complete list of the chemicals used may be given, together with the concentrations and lengths of treatments.

Ethylene chlorhydrin and the thiocyanates of sodium and potassium stood out prominently in these tests, giving the best and most consistent results in forcing the germination of dormant tubers.

Dichloroethylene, trichloroethylene, xylol, and carbon bisulfid also gave good, but somewhat less favorable, results. Experimental work with them is being continued.

Ethyl nitrite, furoic acid, methylene chlorid, furfural, bleaching powder, bromoform, and tribromphenol gave favorable results in some cases but not in others. The cause of this irregularity is not known at present.

Thiourea represents a special case. Solutions of it in proper concentration consistently caused the development of more than one bud per eye, sometimes as many as eight, and in addition caused the sprouting of more than one eye on each seed-piece.

So far as I know, this is the first report regarding the effect of the above-

mentioned substances upon the earliness of sprouting of potato tubers. The use of the following chemicals for this purpose was suggested in 1909 by McCallum (1): ethyl bromid, ethylene dichlorid, carbon tetrachlorid, ammonia, bromine, and gasoline. His experiments with these substances were repeated and favorable results were obtained with the first three, especially with ethylene dichlorid and ethyl bromid. Rosa (2) recommended the use of solutions of sodium nitrate. These were tried in many experiments, and under certain conditions good results were obtained.

### Results with Ethylene Chlorhydrin ( $\text{ClCH}_2\text{CH}_2\text{OH}$ )

Of all the chemicals tried, ethylene chlorhydrin gave the greatest promise of successful practical application for breaking the dormancy of potatoes. Since it is a volatile substance that mixes with water in all proportions, treatments were made either by soaking in solution or by exposure to vapors.

With Bliss Triumph, soaking cut tubers for one hour in solutions varying in concentration from 10 cc.<sup>2</sup> per liter to 3 cc. per liter hastened sprouting (Pl. X, fig. 1). Freshly dug Irish Cobblers required about 4 cc. per liter for two hours.

The volatility of ethylene chlorhydrin permitted its application as a vapor by three different methods: first, by placing on top of the tubers in closed containers shallow pans from which the ethylene chlorhydrin evaporated. In this case, cut tubers required about 16 hours' treatment with an amount of ethylene chlorhydrin equivalent to about 0.5 cc. for each liter of space in the vessel. The results shown in figure 2 were obtained with Bliss Triumph, 1925 crop, dug July 22, 1925, treated and planted August 11, 1925, photographed October 6, 1925.

It was found possible to treat the whole tubers by this method, the requirement being 0.5 to 1.0 cc. ethylene chlorhydrin per liter of space in the container for a period of twenty-four hours.<sup>3</sup> Figure 3 shows the results with Irish Cobbler, freshly harvested 1925 crop. Lot A received 10 cc. ethylene chlorhydrin in a 17.5-liter container for 24 hours. Lot B received no treatment. Second-crop young tubers developed on the vines from the treated lot before any sprouts of the untreated lot appeared above ground. The percentage germination was 65.

Experiments are being carried out to determine whether the effect of this treatment will be retained in the tuber during subsequent storage and, if so, for what storage period. The preliminary results indicate that the effect of the treatment holds over in the tuber during storage for at least three weeks.

<sup>2</sup> The ethylene chlorhydrin referred to in this paper is a commercial grade known as "40 percent ethylene chlorhydrin." The concentrations given do not refer to the anhydrous chemical.

<sup>3</sup> Later experiments showed that when whole tubers were treated better results were obtained if the potatoes were allowed to stand in air after treatment for a day before planting.

A second method of applying the ethylene chlorhydrin consisted in placing the potatoes in containers that could be closed, arranging the tubers in layers about six inches deep, covering each layer with burlap saturated with a solution of ethylene chlorhydrin, closing the container and letting it stand over night (about 16 hours). The concentration of ethylene chlorhydrin used to saturate the burlap cloth was 100 cc. per liter. The results obtained varied from top to bottom of the container, indicating that this method gave an uneven distribution of the vapor inside the container. Further experiments may remedy this defect.

The third method consisted in dipping the potatoes into a solution of ethylene chlorhydrin, removing them at once and placing them in closed containers for about 16 to 24 hours. A uniform amount of liquid was thus distributed over the surface of the potatoes. The ethylene chlorhydrin evaporated, producing within the vessel a vapor, the concentration of which varied with the amount of chemical in solution. With cut tubers a dipping solution containing about 30 cc. of ethylene chlorhydrin per liter gave good results. Sprouts were visible at the surface of the tuber seven days after treatment, and, when planted two inches deep in flats indoors, the plants appeared above ground in another week. In the one series of experiments carried out in this way, uniform results were obtained at the top and bottom of the container. However, further work will be necessary to determine the relation of the size and shape of the container to the problem of obtaining a uniform distribution of vapor.

### Results with Sodium and Potassium Thiocyanate ( $\text{NaCNS}$ and $\text{KCNS}$ )

Sodium and potassium thiocyanate solutions gave notably good results, although the concentration margin between stimulation and injury was not large. With cut tubers in deeply dormant condition, soaking for one hour in 3-percent or 2-percent solutions forced early sprouting of Irish Cobbler and Bliss Triumph, but 1 percent was noticeably less effective and 4 percent caused loss by rotting. For tubers in the latter part of the rest period, 3 percent for one hour was found to be too strong, but 1 percent for one hour was favorable, as was also 0.25 percent for two hours. The results with  $\text{KCNS}$  are shown in figure 4, Plate XI; lot *B* shown in this figure was treated with 2 percent  $\text{KCNS}$  for one hour, lot *A* with  $\text{H}_2\text{O}$  for one hour, and lot *C* with 0.5  $M$   $\text{NaNO}_3$  for one hour. The variety used was Irish Cobbler, Long Island 1925 crop, treated and planted July 21, 1925, photographed September 20, 1925.

### Results with Thiourea ( $\text{NH}_2\text{CSNH}_2$ )

Solutions of thiourea caused the development of more than one bud per eye, and more than one eye per seed-piece. Figure 5 shows the results obtained by soaking cut potatoes in 1-percent thiourea for one hour. In other experiments, not illustrated in this paper, as many as eight buds

developed from one eye. The uniformity with which this result was produced is shown by the fact that, out of 20 pieces treated, 18 formed two or more buds at each eye. The concentration necessary to cause this multiple-bud development varied from about 4 percent for dormant buds to about 1 percent for buds just beginning to sprout.

Solutions of thiourea, therefore, overcame the inhibition which the main bud exercises over the subsidiary buds at each eye and also partially nullified the capacity of the apical bud to prevent the growth of basal buds. It is planned to give further details on this point in a paper that is to follow.

#### Results with Ethylene ( $C_2H_4$ )

Ethylene was used in concentrations varying from 75 percent to one part in five millions for periods varying from one hour to seven days. The results were not encouraging, and in no case did the stimulation produced compare favorably with that caused by some of the other chemicals used. Rosa (3) reported that when the ethylene treatment was continued for a longer time, in his experiment for one month, the dormant period of the tubers was markedly shortened.

#### Results with Sodium Nitrate ( $NaNO_3$ )

With potatoes that were not very dormant, 0.5  $M$   $NaNO_3$  solution for one hour generally gave good forcing action, but the range of favorable concentration was small, 0.3  $M$  solution being ineffective in all cases. Some injury was caused by 0.5  $M$  solution for one hour when used with potatoes late in the dormant period. The sprouting of recently dug Bliss Triumph was forced by one hour's treatment with 0.6  $M$ , but 0.4  $M$  gave results that were little better than those of the check lot. Freshly harvested Irish Cobblers did not respond to treatment for one hour in 0.7  $M$ , 0.5  $M$ , or 0.3  $M$  sodium nitrate.

#### Results with Other Chemicals

Favorable results were obtained with the following chemicals, using the given concentrations for 16 hours with cut tubers, and for 24 hours with whole tubers:

Ethyl bromid, 2 cc. in a 17.5-liter space with cut tubers, and 3 cc. in a 17.5-liter space with whole tubers; ethylene dichlorid, 1 cc. in a 17.5-liter space with cut tubers, and 2 cc. in a 17.5-liter space with whole tubers (the results with ethylene dichlorid are shown in figure 6); carbon bisulfid, 1 cc. in 35-liter space with cut tubers, and 2 cc. in 35-liter space with whole tubers; dichloroethylene, 3 cc. in 17.5-liter space for cut tubers, 8 cc. in 17.5-liter space for whole tubers; trichloroethylene, 2 cc. in 35-liter space for cut tubers, 5 cc. in 35-liter space for whole tubers.

### Concentration of Chemicals as Related to Stage of Dormancy

It was found that concentrations favorable for treating freshly harvested tubers caused injury to tubers that were out of the dormant period and ready for immediate sprouting. The concentrations referred to in this paper are those that were found satisfactory for potatoes treated within about one month after digging. The concentrations given will serve to guide experimenters who wish to make tests with any of these chemicals. In such experiments it will be well to try concentrations stronger and weaker than those mentioned.

### COST OF TREATMENTS

If a practical method could be devised for using these chemicals for hastening the germination of dormant potatoes, their cost would not be prohibitive. Thus, ethylene chlorhydrin (40 percent commercial grade such as used in these experiments) costs 30 cents per pound. If it were used at the concentrations found favorable for forcing potatoes, it is estimated that the cost of the treatment would be approximately 4 cents per bushel; the estimate for trichloroethylene is 1 cent per bushel, and for ethylene dichlorid  $\frac{1}{3}$  cent per bushel.

### SUMMARY

The experiments were undertaken for the purpose of finding a method of hastening the germination of dormant potato tubers.

Two hundred twenty-four different chemicals were tested, and about 3,000 separate experimental lots were used in selecting suitable concentrations and periods of treatment.

The methods used consisted either in soaking the tubers (both cut and whole), usually for one hour, in aqueous solutions of different chemicals of gradually increasing concentrations, or in exposing them for varying lengths of time in closed containers to the vapors from numerous volatile compounds.

It was found that the vapors of ethylene chlorhydrin ( $\text{ClCH}_2\text{CH}_2\text{OH}$ ) were remarkably effective in causing prompt germination of dormant potatoes. With Irish Cobbler, 1925 Long Island crop, the treated lot produced vines two feet high, bearing second-crop tubers 1 cm. in diameter before the checks appeared above ground. Further work is being done to determine whether a standardized procedure for applying the ethylene chlorhydrin treatment can be worked out. There can be no doubt of its effectiveness. Only its practicability is now in question.

Sodium and potassium thiocyanate solutions gave excellent results in forcing early sprouting. Irish Cobbler, Long Island 1925 crop, treated and planted July 21, 1925, gave 100 percent germination. Sprouts appeared above ground one month after planting and in two months produced plants 24 inches high on which young tubers were beginning to form. The un-

treated tubers at this time had not yet appeared above ground. There is some doubt at present whether there is sufficient range of concentration between injury and effectiveness in order that a successful commercial method can be developed. Further experiments on this point are being made.

Other chemicals that gave favorable results were: dichloroethylene, trichloroethylene, carbon bisulfid, ethylene dichlorid, xylol, and ethyl bromid. Tests with these are being continued.

Thiourea represented a special case. Solutions of it in proper concentration not only forced prompt sprouting, but consistently caused the development of more than one sprout per eye, sometimes as many as eight, and in addition induced the development of more than one eye on each potato. It thus overcame the inhibiting effect which the main bud in each eye exerts upon the subsidiary buds, and also partially nullified the capacity of the terminal bud to prevent the development of basal buds on the same seed-piece.

The results with ethylene were unsatisfactory. In no case tried were its stimulating effects comparable with those of the substances principally referred to in this paper. The periods of exposure to ethylene, however, were for a few hours or days only, and not for the long period (one month) by means of which Rosa (3) obtained favorable results with ethylene.

I wish to express my thanks to Dr. William Stuart of the United States Department of Agriculture for furnishing generous quantities of experimental seed potatoes.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.,  
YONKERS, NEW YORK

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#### DESCRIPTION OF FIGURES

##### PLATE X

FIG. 1. *A*, cut tubers soaked 1 hour in a solution of 10 cc. ethylene chlorhydrin in 1 liter; *B*, soaked 1 hour in water; Bliss Triumph, 1925 crop; dug July 22, treated and planted August 11, photographed October 6.

FIG. 2. *A*, ethylene chlorhydrin, 1 cc. in 2-liter space; cut tubers treated 16 hours; *B*, check, not treated; Bliss Triumph, 1925 crop; dug July 22, treated and planted August 11 and 12, photographed October 6.

FIG. 3. *A*, ethylene chlorhydrin, 10 cc. in 17.5-liter space; whole tubers treated 24 hours; *B*, check, not treated; Irish Cobbler, Long Island 1925 crop; treated and planted July 22 and 23, photographed October 6.

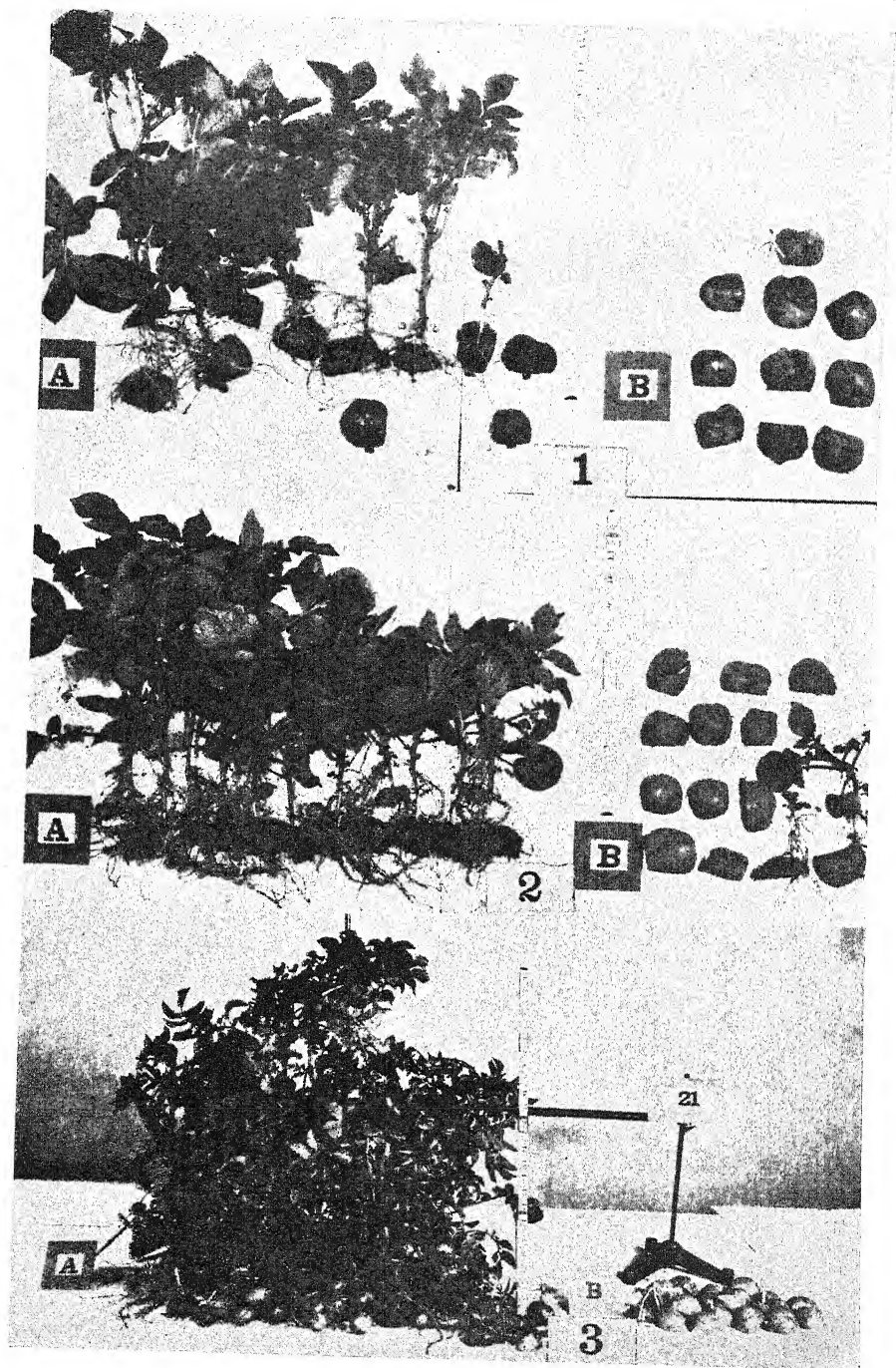


## PLATE XI

FIG. 4. *B*, cut tubers soaked 1 hour in 2-percent solution of potassium thiocyanate; *C*, soaked 1 hour in 0.5 *M* solution of sodium nitrate; *A*, check, soaked 1 hour in water; Irish Cobbler, Long Island 1925 crop; treated and planted July 21, photographed September 20,

FIG. 5. Effect of thiourea in causing the development of more than one bud per eye, and of more than one eye per seed-piece; Rural New Yorker, not dormant, soaked 1 hour in 1 percent thiourea solution, both tubers treated.

FIG. 6. *A*, ethylene dichlorid, 0.5 cc. in 17.5-liter space; cut potatoes treated 16 hours; *B*, check, not treated; Bliss Triumph, 1925 crop; dug July 22, treated and planted in field August 11 and 12, photographed October 6.



DENNY: SPROUTING OF DORMANT TUBERS



## GERMINATION OF SEEDS UNDER WATER

TOSHITARO MORINAGA

(Received for publication December 4, 1925)<sup>1</sup>

### INTRODUCTION

It is a well known fact that seeds of many common crops are not only unable to germinate in water, but gradually lose their viability and finally come to death in it (2, 12, 17). According to Shull (15), however, some kinds of seeds can retain their viability for several years under water. Crocker (4) believed that "the discrepancy between these results and those of Mazé is probably best explained by the fact that Shull was working with the seeds of wild plants, while Mazé was dealing with cultivated species."

Kraus (9) studied the germination of *Triticum vulgare*, *Secale cereale*, *Avena sativa*, *Hordeum vulgare*, *Zea Mays*, *Sinapis alba*, *Polygonum Fagopyrum*, *Pisum sativum*, *Cucurbita Pepo*, *Lupinus luteus*, *Vicia Faba*, *Helianthus annuus*, *Brassica rapa*, *Lepidium sativum*, *Vicia sativa*, *Trifolium pratense*, *Hyacinthus candicans*, and *Lathyrus grandiflorus* in tap water. The last seven species germinated in water and also made considerable later growth. *Cucurbita Pepo* and *Lupinus luteus* showed no germination; all others pushed hypocotyls out of testas without any further growth. He also found that aëration of the water or the addition of small amounts of  $H_2O_2$  was helpful for the growth of germinating seeds.

Kinzel (8) and Lehman (10), in their work on the effect of light on germination, and Godlewski (6), in studying anaërobic growth, observed germination of seeds under water. Kinzel worked on many wild-land and marsh-plant seeds. He obtained especially good germination in water with *Hypericum*, *Rhododendron*, *Azalea*, *Calluna*, *Primula*, *Verbascum*, *Mimulus*, *Digitalis*, *Veronica*, *Campanula*, etc.

In the first part of this work the writer reports the results of a comparison of germination in water and on filter paper. Many different species were included in the study in order to determine whether there is any phylogenetic relation in ability to germinate under water. In the latter part, attention is given to the effect of temperatures on the germination under water.

Nearly all the seeds for the experiment were bought from the Vaughan Seed Company and Stumpp and Walter Seed Company in New York, and were of the crop of 1923.

<sup>1</sup> Published, at the expense of the Boyce Thompson Institute for Plant Research, out of the order determined by the date of receipt of the manuscript.

## EXPERIMENTAL RESULTS

## Comparison of Germination under Water and on Wet Filter Paper

For germination under water, seeds were kept at the bottom of 100 cc. Erlenmeyer flasks filled with distilled water. As checks, the same number of seeds were put on moistened filter paper in Petri dishes. The flasks were put side by side, and the dishes were placed in piles in a greenhouse. The temperatures in the greenhouse varied from 15° to 33° C. The water in the flasks was renewed once in two weeks. The germination record was taken once a week, and seeds in the water were counted as germinated only when hypocotyls or sometimes plumules (epicotyls) also started healthy growth. The experiment was continued for 42 days. The results are summarized in tables 1A-1C.

TABLE 1A. *Seeds Giving no Germination under Water*

Names	No. Seeds per Cul- ture	Percent Germi- nation in Petri Dish	Percent Germi- nation under Water
<i>Festuca pratensis</i> (meadow fescue) . . . . .	100	96.5*	0
<i>Festuca elatior</i> (fall fescue) . . . . .	100	86.5	0
<i>Lolium perenne</i> (English rye grass) . . . . .	100	52.0	0
<i>Andropogon sorghum</i> (common sorghum) . . . . .	25	82.0	0
<i>Avena sativa</i> (oats, without glumes) . . . . .	20	100.0	0
<i>Triticum vulgare</i> (Marquis wheat) . . . . .	25	98.0*	0*
<i>Zea Mays</i> (field corn) . . . . .	5	100.0	0
<i>Coix lacryma</i> (Job's tears) . . . . .	4	100.0	0 (0)†
<i>Setaria macrochaeta</i> . . . . .	50	97.0	0 (0)
<i>Raphanus sativus</i> (radish) . . . . .	25	72.0	0
<i>Petroselinum hortense</i> (parsley) . . . . .	50	77.0	0 (10)
<i>Brassica oleracea</i> (cabbage) . . . . .	50	86.0	0 (0)
<i>Iberis</i> (candytuft) . . . . .	50	82.0	0 (0)
<i>Cosmos</i> . . . . .	50	87.0	0 (0)
<i>Aster</i> . . . . .	50	65.0	0 (0)
<i>Centaurea</i> . . . . .	25	84.0	0 (0)
<i>Carthamus tinctorius</i> (saffron) . . . . .	10	60.0	0 (0)
<i>Capsicum annuum</i> var. <i>longum</i> (pepper) . . . . .	25	74.0	0 (44)
<i>Solanum melongena</i> (egg plant) . . . . .	25	92.0	0 (66)
<i>Allium Cepa</i> (onion) . . . . .	25	60.0	0 (0)
<i>Asparagus officinalis</i> . . . . .	25	58.0	0 (34)
<i>Cucumis Melo</i> (muskmelon) . . . . .	10	95.0	0 (5)
<i>Cucurbita maxima</i> (squash) . . . . .	5	80.0	0 (0)
<i>Viola tricolor</i> (pansy) . . . . .	100	72.0	0 (0)
<i>Fagopyrum esculentum</i> (buckwheat) . . . . .	20	92.5	0
<i>Salvia officinalis</i> (sage) . . . . .	50	84.0	0 (0)
<i>Hyssopus officinalis</i> (hyssop) . . . . .	100	76.5	0
<i>Berberis Thunbergii</i> . . . . .	20	72.5	0 (0)
<i>Abutilon</i> . . . . .	25	98.0	0
<i>Phlox Drummondii</i> . . . . .	25	60.0	0 (0)
<i>Pisum sativum</i> (Alaska pea) . . . . .	10	100.0	0
<i>Phaseolus</i> (bean) . . . . .	5	90.0	0
<i>Lupinus</i> . . . . .	20	50.0	0 (5)
<i>Medicago sativa</i> (alfalfa) . . . . .	100	94.5	0

\* All these numbers are the means of duplicated tests.

† The number in parentheses in the last column shows the percentage of germination after being taken out of the water and placed on moist filter paper.

TABLE 1B. *Seeds Giving Poorer Germination under Water than on Moist Filter Paper*

Names	No. Seeds per Culture	Percent Germination in Petri Dish	Percent Germination under Water
<i>Alopecurus pratensis</i> (meadow foxtail).....	100†	85.0*	67.0*
<i>Festuca duriuscula</i> (hard fescue).....	100	82.0	9.5
<i>Cynosurus cristatus</i> (crested dog's tail).....	100	48.5	16.5
<i>Bromus brizaeformis</i> (brome-grass).....	10†	100.0	85.0
<i>Eragrostis elegans</i> (love grass).....	100	81.5	66.5
<i>Nicotiana</i> .....	100	84.0	68.0
<i>Lycopersicum lycopersicum</i> (tomato).....	50	71.0	30.0
<i>Salpiglossis</i> .....	100	76.0	38.5
<i>Trifolium repens</i> (white clover).....	100	84.5	66.0
<i>Mimosa pudica</i> (sensitive plant).....	10	90.0	80.0
<i>Ocimum basilicum</i> (basil).....	50	68.0	51.0
<i>Thymus vulgaris</i> (thyme).....	100	63.5	25.0
<i>Satureja hortensis</i> (summer savory).....	100	59.5	5.0
<i>Marrubium vulgare</i> (horehound).....	100	44.5	2.0
<i>Origanum Majorana</i> (marjoram).....	100	87.5	62.0
<i>Dianthus</i> (carnation).....	50	99.0	89.0
<i>Allium porrum</i> (leek).....	50	72.0	24.0
<i>Alyssum</i> .....	100	91.5	63.0
<i>Ruta graveolens</i> (rue).....	50	51.0	15.0
<i>Anthemis nobilis</i> (chamomile).....	100	92.5	86.0
<i>Sesamum orientale</i> (bene).....	50	99.0	21.0
<i>Clematis virginiana</i> .....	25	96.0	58.0
<i>Campanula medium</i> (Canterbury bell).....	100	83.0	52.0
<i>Amaranthus</i> .....	100	94.0	87.0
<i>Hunnemannia</i> .....	50	93.0	1.0

\* All these numbers are the means of duplicated tests.

† Without glumes.

TABLE 1C. *Seeds Showing Good Germination under Water*

Names	No. Seeds per Culture	Percent Germination in Petri Dish	Percent Germination under Water
<i>Phleum pratense</i> (timothy).....	100†	93.0*	90.0*
<i>Axonopus compressus</i> (carpet grass).....	100	89.5	93.5
<i>Agrostis nebulosa</i> .....	100†	91.5	96.0
<i>Poa compressa</i> (Canadian blue grass).....	100†	84.5	94.5
<i>Cynodon dactylon</i> (Bermuda grass).....	100†	79.0	95.5
<i>Lactuca sativa</i> (lettuce).....	50	95.0	95.0
<i>Artemisia Absinthium</i> (wormwood).....	100	85.0	89.0
<i>Daucus Carota</i> (carrot).....	25	68.0	66.0
<i>Apium graveolens</i> (celery).....	100	92.0	88.0
<i>Portulaca</i> .....	100	78.0	81.0
<i>Mesembryanthemum crystallinum</i> (ice plant).....	100	82.0	87.0
<i>Antirrhinum</i> (snapdragon).....	100	88.0	92.0
<i>Melissa officinalis</i> .....	100	80.0	74.5
<i>Celosia</i> (cockscomb).....	100	94.5	94.5
<i>Oenothera</i> (evening primrose).....	100	85.0	84.0
<i>Silene latifolia</i> .....	100	97.5	94.5
<i>Solanum Dulcamara</i> (bittersweet).....	50	99.0	98.0
<i>Petunia</i> .....	100	93.5	91.5

\* All these numbers are the means of duplicated tests.

† Without glumes.

When, instead of 100, only 10 hulled grains of *Festuca pratensis*, *Festuca elatior*, *Lolium perenne*, and only 10 seeds of *Medicago sativa* were taken in each flask, they germinated 25.5, 0.5, 14, and 95 percent respectively, thus showing an increased germination under water when a smaller number of seeds per flask were taken.

These experiments are considered preliminary especially on account of the uncontrolled temperature and light conditions.

Some unfavorable conditions obtain in the water cultures. Thus, the great depth of water makes oxygen inaccessible (9); furthermore, distilled water was used exclusively and renewed only once in two weeks. Out of 78 genera of 24 families, 43 genera germinated in the water. Even some of the 35 genera which did not germinate under the conditions of the experiments did germinate if fewer seeds were placed in the flasks, so that the small amount of oxygen present was used by fewer seeds.

Amongst the 43 genera which germinated in the water, 18 genera did not show any decided difference between the germination in the water and that on filter paper, and 2 genera (*Cynodon dactylon* and *Poa compressa*) germinated better under water than on blotters. Egg plant, pepper, and asparagus seeds kept their vitality well during 6 weeks' submersion. The ability to germinate under water obtained more generally for small seeds, and was not related to phylogeny or to the kinds of reserve material in the seeds.

#### Germination of Seeds in Boiled Distilled Water

Hartleb and Stuzer (7) and Kraus (9) maintained that seeds are unable to germinate in boiled water on account of the absence of oxygen. Florence

TABLE 2. Germination of Seeds in Boiled Water

Names	No. Seeds per Flask	Percent Germination	No. Days Tested
<i>Phleum pratense</i> (timothy).....	25	60.0*	9
<i>Cynodon dactylon</i> (Bermuda grass).....	25	86.0	9
<i>Poa compressa</i> (Canadian blue grass).....	25	18.0	11
<i>Festuca pratensis</i> (meadow fescue).....	10	25.0	11
<i>Festuca elatior</i> (fall fescue).....	10	50.0	11
<i>Lactuca sativa</i> (lettuce).....	20	12.5 (poor)	13
<i>Artemisia Absinthium</i> (wormwood).....	25	54.0	5
<i>Anthemis nobilis</i> (chamomile).....	25	76.0	5
<i>Daucus Carota</i> (carrot).....	10	45.0	15
<i>Apium graveolens</i> (celery).....	20	52.5	21
<i>Trifolium repens</i> (white clover).....	20	51.5	5
<i>Medicago sativa</i> (alfalfa).....	10	0	—
<i>Antirrhinum</i> (snapdragon).....	25	60.0	9
<i>Ocimum basilicum</i> (basil).....	10	75.0	9
<i>Celosia</i> (cockscomb).....	25	74.0	7
<i>Dianthus</i> (carnation).....	20	70.0	7
<i>Solanum Dulcamara</i> .....	20	87.5	11
<i>Sesamum orientale</i> (bene).....	10	55.0	15
<i>Alyssum</i> .....	20	67.5	7
<i>Petunia</i> .....	25	78.0	9
<i>Mesembryanthemum crystallinum</i> (ice plant)	20	45.0	7

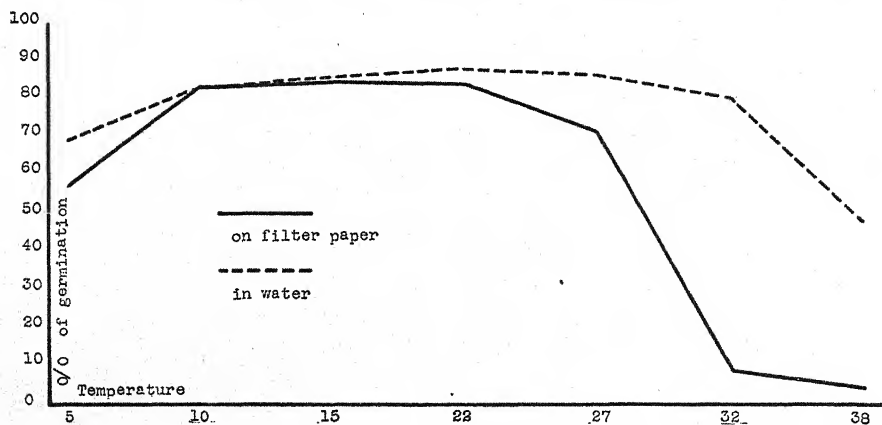
flasks of 100-cc. capacity were nearly filled with boiled distilled water and cooled quickly in ice water. As soon as the water was cool enough, seeds were put in and sealed with paraffin oil. These flasks were placed in the greenhouse as experiment *A*. When the seedlings that germinated in the flask became green, records were taken and the flask was discarded because oxygen could be liberated inside the flask by photosynthesis as soon as chlorophyll was formed. This experiment, therefore, does not show any final percentage of germination, but shows whether seeds can germinate at all in this condition. Twenty-one kinds of seeds which germinated well in water in experiment *A* were chosen for this experiment. The results obtained are shown in table 2.

These results do not agree with those of the workers above mentioned, the seeds of all species except alfalfa giving either fair or good germination. This discrepancy, however, may have been due to a difference in the method of sealing, to a difference in species, or to a more favorable alternation of temperature. Carnation, chamomile, wormwood, timothy, white clover, and Celosia seeds were chosen and tested for germination in Petri dishes in a sealed jar containing potassium pyrogallate for absorbing oxygen. None of these germinated.

#### Germination of Seeds in Water in Contact with Pure Oxygen

Running water (2), artificial aëration, or addition of  $H_2O_2$  (9, 12) to increase dissolved oxygen was successfully used to germinate seeds in water. In this experiment only seeds which did not germinate in experiment *A* (see table 1A) were chosen and tested for germination in the water. They were placed in contact with pure oxygen instead of with air.

Small numbers of seeds were put in 500-cc. Erlenmeyer flasks with 50 cc. distilled water, the depth of immersion being about one centimeter.



TEXT FIG. 1. Curves showing germination of white clover seeds in water and on filter paper at various temperatures; curves plotted from table 3.



In one flask of each pair the air inside was replaced with pure oxygen. Common sorghum, wheat, oats, corn, Alaska pea, onion, cabbage, muskmelon, kohlrabi, and mustard were tried. Mustard, kohlrabi, cabbage, oats, and common sorghum germinated in both conditions, and wheat, onion, and muskmelon germinated only in the oxygen flask. They all grew well and made green seedlings. Corn and pea did not germinate in either the air or the oxygen flask.

From this experiment it is clear that a number of the seeds listed in table 1A as showing no germination under water can germinate in water if the oxygen supply is increased.

### Effect of Temperature on Germination under Water

According to Akemine (1), the seeds of *Oryza sativa* germinated 94 percent at 40° C. in water, but none of them germinated on wet filter paper at the same temperature. The seeds, however, germinated as well on filter paper as in water at the optimum temperature (30°–35° C.), and again germinated better in water at the minimum (10°–12° C.).

We have observed similar results with white clover seeds, and wish to emphasize the importance of temperature effects when a comparison is made between germination in water and germination on filter paper.

To avoid other factors which affect the germination, especially in water, only 10 seeds were put with 100 cc. distilled water in 100-cc. Erlenmeyer flasks, or on filter paper in Petri dishes. The water in the flasks was not changed during the experiment. The results are shown in table 3 and in text figure 1.

TABLE 3. Germination of White Clover Seeds

Temperature	No. Seeds Tested	Percent Germination in Water			No. Seeds Treated	Percent Germination on Wet Filter Paper		
		After 2 Days	After 6 Days	After 10 Days		After 2 Days	After 6 Days	After 10 Days
5°	10 x 10	0	43	69*	10 x 10	0	13	57†
10°	10 x 10	37	83	83	10 x 10	13	83	83
15°	16 x 10	71.3	85.6	86.3	16 x 10	56.3	83.8	85.0
22°	10 x 10	82	87	88	10 x 10	69	83	84
27°	10 x 10	81	87	87	10 x 10	54	72	72
32°	16 x 10	69.4	79.4	81.3	16 x 10	6.3	8.1	8.8
38°	10 x 10	37	47	49	10 x 10	1	1	4

\* 76 percent germination after 14 days.

† 72 percent germination after 14 days.

As is shown in table 3 and in text figure 1, high temperature retards germination markedly in Petri dishes, but much less in water. At 15° C. or at lower temperatures, there was practically no difference between the water and the filter paper; but when the temperature was 32° C. or higher, nearly ten times as many seeds germinated in water as in Petri dishes. This was not merely a response of a special variety or lot of white clover

seeds, since three additional lots purchased from three different seedsmen gave the results, as shown in table 4.

TABLE 4. *Germination of White Clover Seeds*  
(10 x 10 seeds of each lot were tested for each temperature;  
the water in the flasks was not renewed)

*Germination Test at 32° C.*

	In Water		On Wet Filter Paper	
	Percent Germination after 10 Days	Percent Hard Seeds	Percent Germination after 10 Days	Percent Hard Seeds
Lot B. ....	68	4	10	5
Lot C. ....	58	12	11	4
Lot D. ....	69	12	20	13

*Germination Test at 15° C.*

	In Water		On Wet Filter Paper	
	Percent Germination after 10 Days	Percent Hard Seeds	Percent Germination after 10 Days	Percent Hard Seeds
Lot B. ....	89	11	87	12
Lot C. ....	79	10	75	16
Lot D. ....	81	14	85	14

Several other experiments were run in Petri dishes at 32° C. to test the effect of various substrata upon the percentage of germination. Table 5 shows the results of these.

TABLE 5. *Germination of White Clover Seeds in Petri Dishes at 32° C.*

Conditions	No. Seeds Tested	Per cent Germination after 10 Days
Germination on filter paper.....	16 x 10	8.8
Germination between filter paper.....	4 x 50	8.5
Germination on filter paper in dishes, keeping NaOH container inside.....	2 x 100	8
Germination on filter paper, without cover of dish.....	2 x 100	1
Germination in quartz sand, in Petri dishes.....	2 x 100	13
Germination in river sand, in Petri dishes.....	2 x 100	39
Germination on filter paper, in Petri dishes.....	2 x 100	17.5
Germination on filter paper, with $\frac{N}{100}$ KNO <sub>3</sub> .....	2 x 100	6
Germination on potting soil, in Petri dishes.....	2 x 100	32
Germination on filter paper, first 6 hours at 5° C.....	2 x 100	5
Germination on filter paper, first 6 hours at 10° C.....	2 x 100	3.5
Germination on filter paper, first 6 hours at 15° C.....	2 x 100	3
Germination on filter paper, seeds injected with water*.....	2 x 100	5.5
Germination on filter paper, seeds sealed with wax.....	2 x 200	44
Germination on filter paper, dishes sealed with wax keeping NaOH container inside.....	2 x 200	4

\* The seeds were injected by removing and replacing the air pressure repeatedly while the seeds were under water.

None of these conditions gave germination equal to that in water.

### Effect of Seed Coat upon Germination of White Clover Seed at High Temperature

Since the rôle of seed coats in germination was worked out by Crocker (3) in 1906, many experimenters dealing with seeds giving poor germination have paid special attention to seed-coat effects.

Several experiments were made to determine whether the seed coats of white clover are a factor in the failure of germination on wet filter paper at high temperatures. Treatment of white clover seeds with concentrated sulfuric acid increases the percentage of germination on filter paper at 32° C.; 20 minutes' treatment gave a germination of 51.5 percent within 10 days on filter paper at 32° C. The portion of the seed coat where the hypocotyl breaks through was cut off with a sharp razor, and the seeds thus treated were kept at 32° C., half in water and half on filter paper. After the treatment they gave 94 percent germination in water, and 31 percent on blotters. After keeping seeds on filter paper at 32° C. for 1 day, the coats were carefully removed. The naked embryo thus obtained germinated 100 percent in water at 32° C., and 73.3 percent on blotters at the same temperature. The 26.3 percent which did not germinate on blotters germinated when they were transferred into water.

From these experiments it is seen that treatment or removal of the seed coat is effective in increasing the germination of white clover seeds on filter paper at 32° C., but better results are always obtained in water with the seeds treated in the same way.

### Effect of Varying the Partial Oxygen Pressure

Several experiments were carried out under various conditions of reduced oxygen supply. It has already been shown in table 2 that white clover seeds will germinate in the greenhouse in boiled water sealed with paraffin oil. Using 10 seeds in each Florence flask, these experiments were repeated at 32° C. and 15° C., 6 percent germinating at 32° C. and 74 percent at 15° C.

In order to learn whether the seed will germinate in total absence of oxygen as observed by Takahashi (16) with *Oryza sativa*, Crocker and Davis (5) with *Alisma Plantago*, and Godlewski (6) with *Lupinus* in sugar solution, 10 seeds were placed with 10 cc. of distilled water into a 25-cc. distilling flask. The mouth of the flask was sealed just above the side tube. The side tube was then drawn out to a capillary in one region, and connected with a high vacuum. The flask was exhausted and kept under water at 40° to 45° C. for 30 minutes, and the side tube was sealed while still under this condition. During this time the water in the flask was reduced from 10 cc. to 1 cc.; the contents of the flask were always cool because of the rapid evaporation of water. None of the seeds treated in this way germinated at 15° C., the best temperature for germination of white clover.

Germination was also tried in reduced partial oxygen pressure obtained by mixing nitrogen or hydrogen with air. One or two Petri dishes containing 100 seeds each were put on a tripod standing in a pan of water. A battery jar was inverted over the Petri dish of seeds and the tripod. This gave a water-sealed air chamber within the battery jar; 60 percent of the air inside the jar was withdrawn and replaced by the same amount of nitrogen or hydrogen.

TABLE 6. *Germination of White Clover Seeds in Hydrogen or Nitrogen Gas Mixture at 32° C.*

Conditions	Percent Germination after			
	2 Days	4 Days	6 Days	8 Days
Air.....	43*	50.5	51	51.5
60 percent N <sub>2</sub> .....	24	43	46	47
60 percent H <sub>2</sub> .....	52	67.5	69.5	70

\* These figures are the mean values of duplicated tests.

Table 6 shows that the germination of white clover was increased when the oxygen concentration of the air was reduced by mixing with it 60 percent of hydrogen. No increase in germination was obtained, however, when nitrogen was used as the diluent. The reasons for this difference between the effects of hydrogen and of nitrogen are not known.

The experiment with seeds in 60-percent H<sub>2</sub> gas mixture with air at 32° C. was repeated 9 times with 1,300 seeds under the same or slightly modified conditions. The average germination was 61.2 percent. The experiment with the control lot exposed to air at 32° C. was repeated 15 times with 2,600 seeds. The average germination was 36.1 percent. For a check on the above described experiments, germination was tested 15 times in Petri dishes with 1,500 seeds with an average germination of 8 percent.

This series of experiments was also carried out at 15° C. and at 38° C. At 15° C. the seeds germinated equally well in all conditions. At 38° C. they germinated better in H<sub>2</sub> mixture, although the percentage of germination was low. The seedlings germinated in H<sub>2</sub> gas mixture were stunted, but they were normal in N<sub>2</sub> gas mixture.

#### General Considerations on Germination of White Clover Seeds

White clover seeds germinate at temperatures between 10° and 38° C., the optimum temperature being about 15° C. The demand for oxygen for the germination of the seed is very low and dissolved oxygen in water is sufficient for germination, so that the statement of Schaible (14) that seeds germinate more slowly and in fewer numbers in reduced air medium than in normal air does not seem applicable to white clover or to most of the seeds listed in table 1C. In fact, white clover seeds germinate and

grow in boiled water covered with paraffin oil. They are not, however, able to germinate in a vacuum.

This fact suggests that a slight amount of oxygen in the tissue or in the medium is necessary for germination. This was also the case for *Typha* and *Cynodon* seeds (see paper in the present issue of this JOURNAL on "The favorable effect of reduced oxygen supply upon the germination of certain seeds"), but not for *Oryza sativa*. This amount of oxygen may hardly be enough to supply energy for germination by normal respiration, but may suffice to initiate some chemical change in the seeds. Most of the white clover seeds of the lot tested were not hard. They germinated about 85 percent at 15° C. in water and on wet paper, but at 32° C. and 38° C. the germination is much poorer on filter paper than in water. This difference is much reduced when the naked embryo is used.

What is the rôle of the seed coat in retarding the germination at high temperatures on filter paper? Since enough water for germination is taken by intact seeds at lower temperatures, and since a trace of oxygen is sufficient for germination, the exclusion of oxygen or water by the coat is probably not the chief reason for the failure to germinate. Insufficient diffusion of CO<sub>2</sub> out of the seed through the coat, or simple mechanical obstruction to the increase of the volume of the embryo, also deserves consideration. This idea is not supported by experimental results at present, but may furnish a suggestion for further investigation.

Why does the naked embryo of a white clover seed germinate more poorly on filter paper than in water at high temperatures? To solve this problem, we must know the significance of the maximum temperature for germination, which sometimes is much lower than the maximum temperature for the growth of the seedling. The raising of the temperature increases the amount of respiration, and if respiration becomes so vigorous as to use up all reserve material available at the temperature, the seed will not be able to germinate (11). On the other hand, the increase of respiration caused by the raising of temperature increases the amount of toxic by-products of respiration. If these substances accumulate to a certain amount in the seeds, germination will be retarded. The amount of reserve material (18, 5) and the amount of respiration will be changed by the amount of oxygen available to the seed. The amount of toxic substances is also related to oxygen supply, and if the seed starts anaërobic respiration, these substances will become more abundant (13). If such conceptions are reasonable, we can imagine the change of maximum temperature according to the amount of oxygen in the medium for germination. Whether the increase of oxygen raises the maximum temperature or decreases it may depend not only upon the reserve materials and the construction of the seed, but also upon the character of the protoplasm itself. Without a study of the chemical changes actually going on in these germination media at high and low temperatures, speculation regarding the reason for the

better germination in water than on wet paper at high temperatures is of little value.

### Germination of Sweet Clover (*Melilotus*) Seeds

Ten previously filed seeds were placed in 100-cc. Erlenmeyer flasks with 100 cc. distilled water, or on filter paper in Petri dishes. One set out of two was kept at 15° C., and the other at 32° C. The results are given in table 7.

TABLE 7. *Germination of Sweet Clover Seeds*

Temperature	Condition	No. Seeds Tested	Percent Germination and Hard Seeds after									
			2 Days		4 Days		6 Days		8 Days		10 Days	
			Germ.	H.S.	Germ.	H.S.	Germ.	H.S.	Germ.	H.S.	Germ.	H.S.
32° C.	In water	10 x 10	30	3	73	3	88	3	88	3	89	3
	On paper	10 x 10	5	4	17	3	54	3	75	3	87	3
15° C.	In water	10 x 10	90	8	94	6	95	4	96	4	96	3
	On paper	10 x 10	98	2	98	1	99	1	99	1	99	1

In this case, there was no difference between the final percentages of germination in water and on filter paper, but at 32° C. the seeds germinated much more quickly in water than on filter paper.

### Germination of Red Clover (*Trifolium pratense*) and Alfalfa (*Medicago sativa*) Seeds

These seeds were tried without filing in water and on filter paper in the same way as other white or sweet clover seeds. The results are given in table 8.

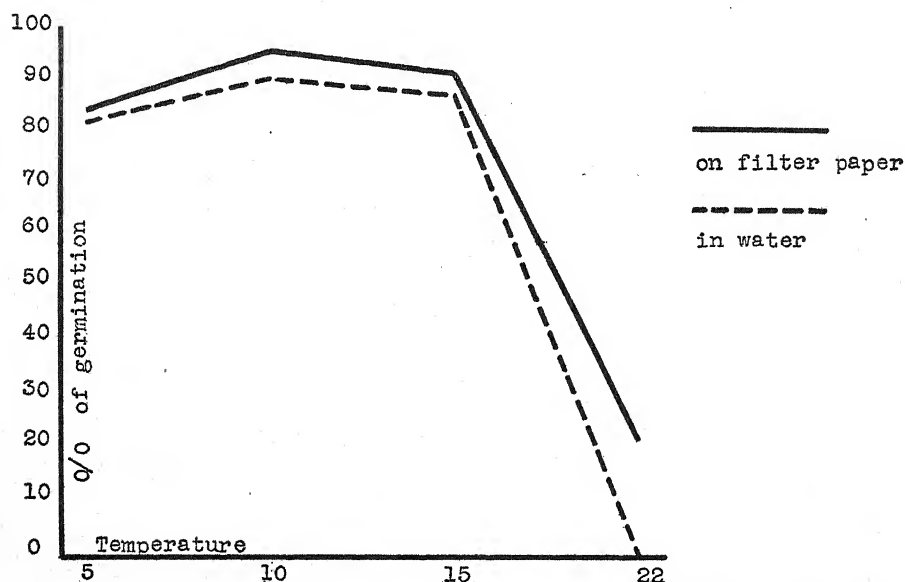
TABLE 8. *Germination of Red Clover and Alfalfa Seeds*

Kind of Seeds	Temperature	Number of Seeds Tested	Percent Germination and Hard Seeds after 10 Days			
			In Water		On Paper	
			Germ.	H.S.	Germ.	H.S.
Red clover.....	15° C.	10 x 10	88	6	87	10
	32° C.	10 x 10	68	5	72	6
	38° C.	10 x 10	3	2	58	8
Alfalfa.....	15° C.	10 x 10	90	2	93	1
	32° C.	10 x 10	49	2	82	0

From table 8 it can be seen that the results with red clover and alfalfa differed from those obtained with sweet clover in one important respect, namely, germination in water was *not better* than germination on filter paper at temperatures above the optimum.

Germination of Celery (*Apium graveolens*) Seeds

Seeds were placed in 100-cc. Erlenmeyer flasks with 100 cc. distilled water, or on filter paper in Petri dishes. The flasks and dishes were wrapped with three sheets of black paper to avoid the favorable effect of light on germination at high temperatures,<sup>2</sup> and kept in thermostats at various temperatures. The water in the flasks was renewed once after 14 days. The results are shown in table 9 and text figure 2.



TEXT FIG. 2. Curves showing germination of celery (var. Schumacher) in water and on filter paper at various temperatures; curves plotted from table 9.

TABLE 9. Germination of Celery Seeds

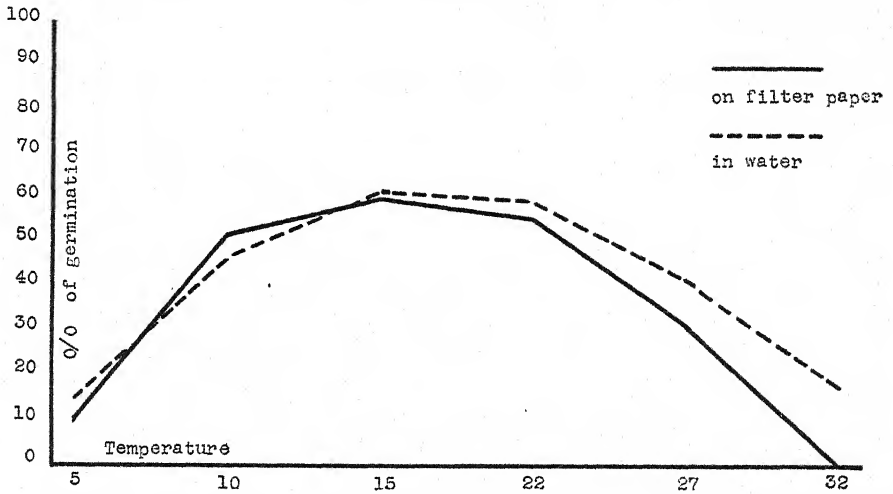
Variety Name	Temperature	No. Seeds Tested	In Water					On Paper				
			Percent Germination after (Weeks)					Percent Germination after (Weeks)				
			2	4	6	8	10	2	4	6	8	10
Dreer's Monarch..	5° C.	2 x 100	0	0	1.0	17.5	35.0	0	0	2.0	21.5	27.5
	10° C.	2 x 100	0	19.5	55.0	62.0	65.0	0	36.5	53.5	61.5	63.0
	15° C.	2 x 100	0	34.0	63.0	67.5	67.5	14.0	50.0	56.5	58.5	59.5
	22° C.	2 x 100	0	0	0	0	0	1.0	1.0	1.0	1.0	1.0
	27° C.	2 x 100	0	0	0	0	0	0	0	0	0	0
Schumacher.....	5° C.	2 x 100	0	0	4.0	40.5	81.5	0	0	60.5	83.5	84.0
	10° C.	2 x 100	0	13.5	66.5	88.0	89.5	4.0	91.0	94.5	94.5	94.5
	15° C.	2 x 100	0	9.5	76.5	86.0	86.5	48.0	86.5	90.0	91.0	91.0
	22° C.	2 x 100	0	0	0	0	0	6.5	16.0	20.5	20.5	20.5
	27° C.	2 x 100	0	0	0	0	0	0	0	0	0	0

<sup>2</sup> We noticed that the maximum temperature for the germination of celery seeds is raised by the effect of light.

Table 9 and text figure 2 show that celery seeds require more time to germinate in water than on filter paper, but there is no difference in final percentage of germination. One variety germinated slightly better, but the other slightly less in water than on filter paper. The maximum temperature, however, is higher on filter paper.

### Germination of Water Cress (*Roripa nasturtium* Rusby) Seeds

As an additional example of water-plant seeds, the germination of water cress seeds was studied at various temperatures in water and on filter paper. The water in the flask was not changed during the experiment. The results are shown in table 10 and text figure 3.



TEXT FIG. 3. Curves showing germination of water cress seeds in water and on filter paper at various temperatures; curves plotted from table 10.

TABLE 10. *Germination of Water Cress Seeds*

Temperature	No. Seeds Tested	In Water				On Paper			
		Percent Germination after				Percent Germination after			
		4 Days	9 Days	15 Days	21 Days	4 Days	9 Days	15 Days	21 Days
5° C.	2 x 100	0	0	3.5	14.5	0	0	0.5	10.0
10° C.	2 x 100	0	21.5	44.5	47.0	0	18.0	41.0	52.0
15° C.	2 x 100	26.0	57.5	60.0	61.5	13.5	47.0	55.0	59.5
22° C.	2 x 100	35.0	58.5	59.0	59.5	20.0	46.0	53.0	56.0
27° C.	2 x 100	7.5	38.5	42.0	42.0	12.5	20.0	26.5	32.0
32° C.	2 x 100	2.5	10.5	16.5	17.5	0	0	0	0
10-22° C.	2 x 100	2.5	44.0	56.0	57.5	0	54.5	61.5	62.0
10-27° C.	2 x 100	2.5	45.0	54.5	55.5	0	49.0	60.0	62.0
10-32° C.	2 x 100	9.5	54.5	61.0	62.5	0	47.5	59.0	60.5
10-38° C.	2 x 100	0	30.5	47.0	47.5	0	0.5	11.5	16.0
15-27° C.	2 x 100	28.5	51.5	52.5	52.5	28.5	50.5	55.5	57.5
15-32° C.	2 x 100	28.5	54.5	57.5	58.0	24.5	49.5	52.5	53.5
15-38° C.	2 x 100	13.0	50.5	55.0	56.5	0	22.0	34.0	36.5
22-32° C.	2 x 100	36.0	56.5	58.0	58.0	26.0	45.0	50.0	53.0
22-38° C.	2 x 100	26.5	51.5	53.0	54.0	0	20.5	29.5	30.0



Table 10 and text figure 3 show that water cress seeds germinate better in water than on filter paper when the temperature is higher than the optimum. This is in agreement with the observations made on white clover seeds.

#### SUMMARY

1. Out of 78 genera of 24 families, 43 genera germinated in water. Even some of the 35 genera which did not germinate under the conditions of the experiment germinated if fewer seeds were placed in the flask of water.

2. Amongst 43 genera which germinated in the water, 18 genera showed no decided difference between the germination in the water and that on filter paper, and 2 genera germinated better under water than on the paper.

3. The ability to germinate under water obtained more generally for small seeds, and was not related to phylogeny or to the kinds of reserve material in the seeds.

4. Out of 21 kinds of seeds which germinated well in distilled water, 20 kinds germinated in boiled distilled water covered with paraffin oil.

5. Several seeds which did not germinate in water were able to germinate when the water was in contact with pure oxygen instead of with air.

6. White clover (*Trifolium repens*) seeds germinated as well in water as on filter paper at optimum temperature (15° C.), but gave nearly 10 times as large percentage of germination in water as on filter paper at 32° C. and at 38° C.

7. Treatment or removal of seed coats was effective in increasing the germination of white clover seeds on filter paper at 38° C., but better results were obtained in water with the seeds treated in the same way.

8. White clover seeds are able to germinate in boiled water sealed with paraffin oil, but are not able to germinate in a vacuum at the optimum temperature. They germinate better in a sealed air chamber over water than in common Petri dishes at 32° C., and still better germination was observed in 60-percent H<sub>2</sub> mixture with air, but 60-percent N<sub>2</sub> mixture was not as favorable as air.

9. Sweet clover (*Melilotus*) seeds germinate equally well in water and on filter paper at high or low temperatures, but they germinate much more quickly in water at high temperature than on the paper.

10. The seeds of red clover (*Trifolium pratense*) and alfalfa (*Medicago sativa*) germinate equally well at the optimum temperature in water and on filter paper, but germination is poorer in water than on paper at temperatures higher than the optimum.

11. Celery (*Apium graveolens*) germinates more slowly in water, but shows no difference between water and filter paper in final percentage of germination. The maximum temperature, however, is higher on filter paper than in water.

12. The seeds of water cress (*Roripa nasturtium* Rusby) germinate

better in water than on filter paper when the temperature is higher than the optimum ( $15^{\circ}$  C.). These seeds also have a higher maximum temperature in water.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.,  
YONKERS, NEW YORK

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## EFFECT OF ALTERNATING TEMPERATURES UPON THE GERMINATION OF SEEDS

TOSHITARO MORINAGA

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### INTRODUCTION

Beneficial effects of daily alternations of temperatures in hastening and increasing the percentage of germination of seeds of several species of plants have been proved by many investigations. Alternations of temperatures have also been long used for the practical testing of such seeds as respond to these conditions.

It has been shown that nitrogen compounds and light favor the germination of some seeds that respond to alternation of temperatures. These are often most effective if applied in combination with the alternation of temperatures. In some seeds the need of alternating temperatures is entirely imposed by the seed coats, and this need disappears if the coats are broken.

Since Harrington (7) has recently given an excellent review of the literature and a statement of the theories regarding the effects of alternating temperatures upon the germination of seeds, these points need not be discussed further in this paper, except for such points as naturally need consideration in the discussion of results.

The work here reported includes the following extensions of previous work done on the effect of alternating temperatures upon the germination of seeds: a greater variety and range of alternations, especially the use of 5°, 10°, and 15° C. as the lower temperatures; better controlled temperatures; the effect of the alternations under water and in reduced oxygen pressure, as well as on moist substrata in the usual oxygen pressures; studies of the several factors accessory to alternate temperatures; and the use of seeds of *Typha latifolia* and *Berberis Thunbergii*, not before known to respond to alternating temperatures.

### EXPERIMENTAL METHODS

The following seeds were used in the experiments: Bermuda grass (*Cynodon dactylon* (L.) Pers.), Canada blue grass (*Poa compressa* L.), celery (*Apium graveolens* L.), cat-tail (*Typha latifolia* L.), and Japanese barberry (*Berberis Thunbergii* DC.). All the seeds used were harvested in 1923. In many cases the experiments were run both in the spring and in the

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autumn of 1924, so as to observe the effects of the length of the period of dry storage upon germination behavior.

The seeds were placed in constant-temperature ovens regulated to temperatures of  $10^{\circ}$ ,  $15^{\circ}$ ,  $22^{\circ}$ ,  $27^{\circ}$ ,  $32^{\circ}$ , and  $38^{\circ}$  C. The alternations were secured by daily transfer from one constant temperature to another. This means a rather sudden heating or cooling, depending on the transfer made.

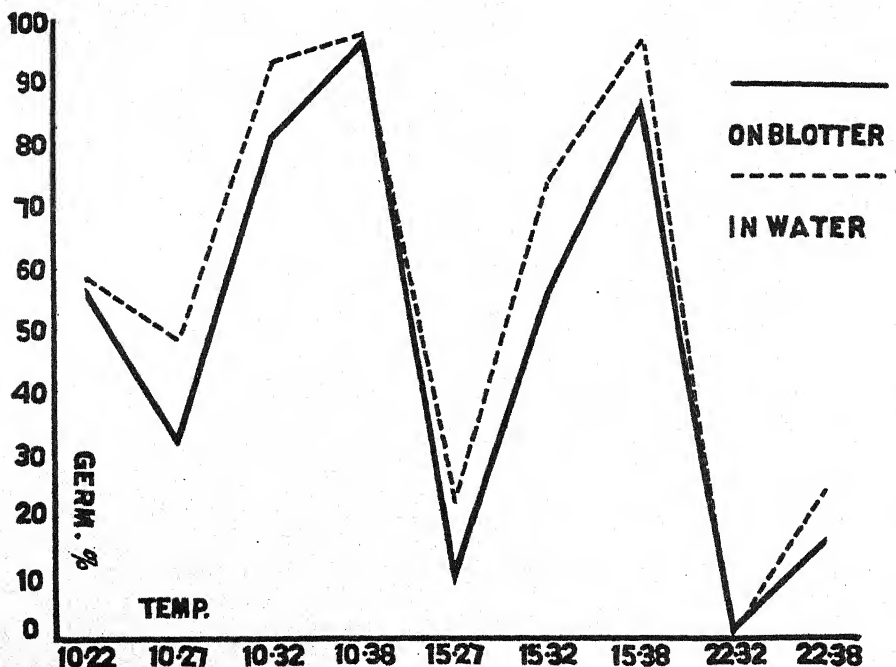
Two types of germination chambers were used: (1) seeds were placed on moist filter paper in Petri dishes, and (2) seeds were kept at the bottom of 100-cc. Erlenmeyer flasks filled with distilled water.

Generally the exposure to the higher temperatures was for 6 hours, and to the lower for 18 hours; but some experiments were carried out to compare the effect of the reverse condition, 18 hours at high temperature and 6 hours at low temperature; and in one experiment a series of duration periods extending from 40 minutes to 8 hours was tried.

### EXPERIMENTAL RESULTS

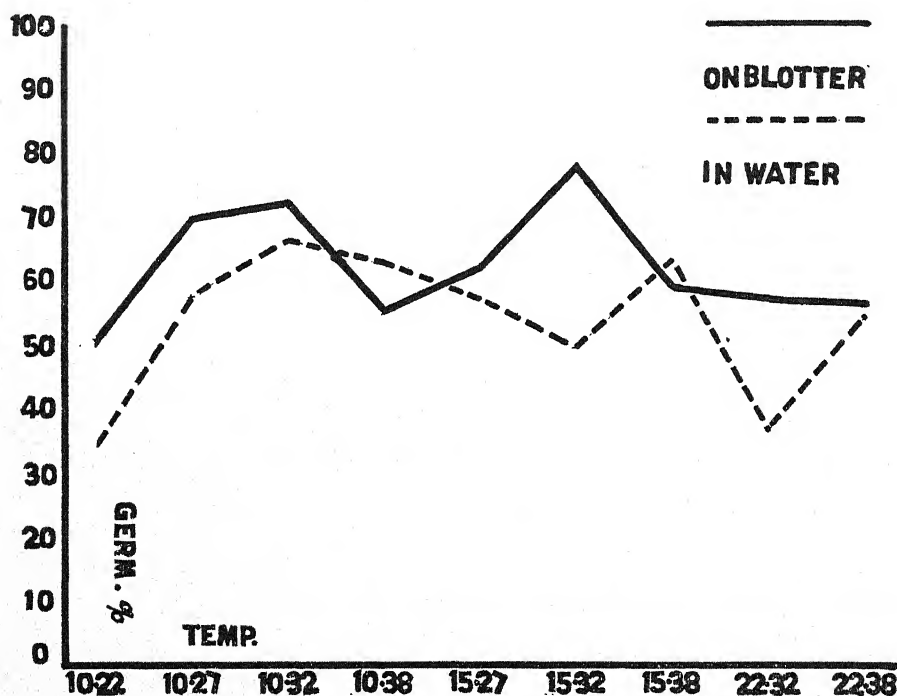
#### Effect of Different Combinations of Alternating Temperatures

On account of lack of space, it was found impossible to include tables showing all the results obtained. In place of them, graphs are herewith presented. The original data can be read with sufficient accuracy from these graphs.



TEXT FIG. 1. Effects of different pairs of alternating temperatures on germination of seeds of Bermuda grass (*Cynodon dactylon* (L.) Pers.).

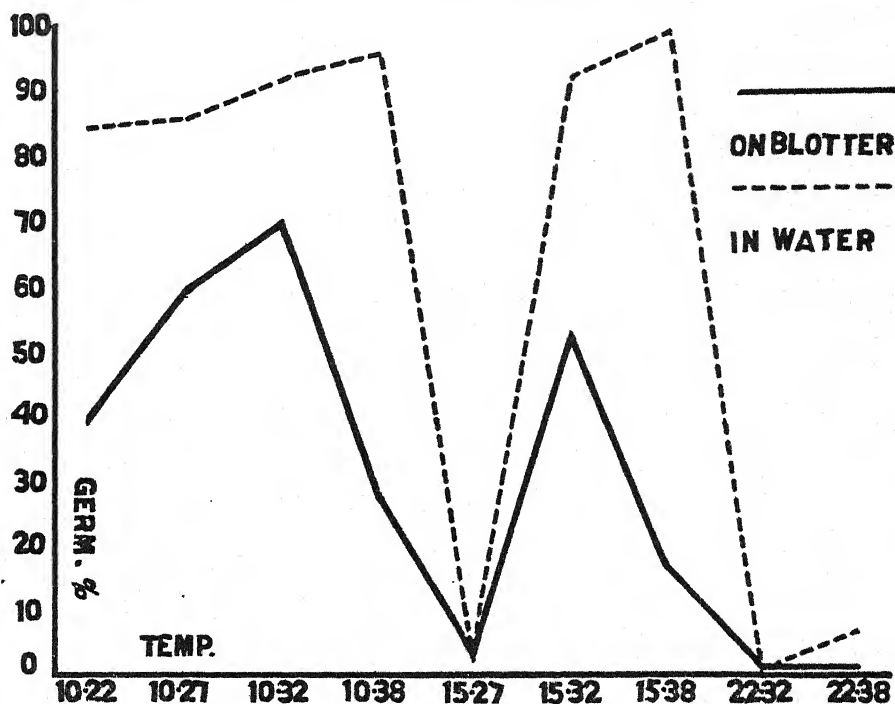
*Bermuda Grass.* Text figure 1 shows the results with nine pairs of alternating temperatures. Bermuda grass does not germinate in constant temperatures in darkness, but it responds well to alternation of temperatures, especially when there is a relatively wide range between the two temperatures used in the daily alternations. Of the nine alternations used with the 6-hour daily period at the higher temperatures,  $10^{\circ}$ – $38^{\circ}$  C. was most effective, and  $10^{\circ}$ – $32^{\circ}$  C. and  $15^{\circ}$ – $38^{\circ}$  C. were slightly less effective. At alternate temperatures Bermuda grass seeds germinate about equally well under water and on moist filter paper. This is probably because these seeds are indifferent to the wide range of oxygen supply represented by these two extremes. As later experiments show, the seeds contain some oxygen and there is some in the distilled water.



TEXT FIG. 2. Effects of different pairs of alternating temperatures on germination of seeds of Canada blue grass (*Poa compressa* L.).

*Canada Blue Grass.* The results are shown in text figure 2. Canada blue grass seeds are not such strict requirers of alternate temperatures in darkness as are Bermuda grass seeds. They gave 12.5 percent germination at  $22^{\circ}$  C. constant, while the best alternations gave only 77.5 percent. The alternation  $15^{\circ}$ – $32^{\circ}$  C. was very effective. The alternation  $15^{\circ}$ – $38^{\circ}$  C. was also effective, giving better germination in one case than other alternations, although  $38^{\circ}$  C. has previously been supposed to be too high for

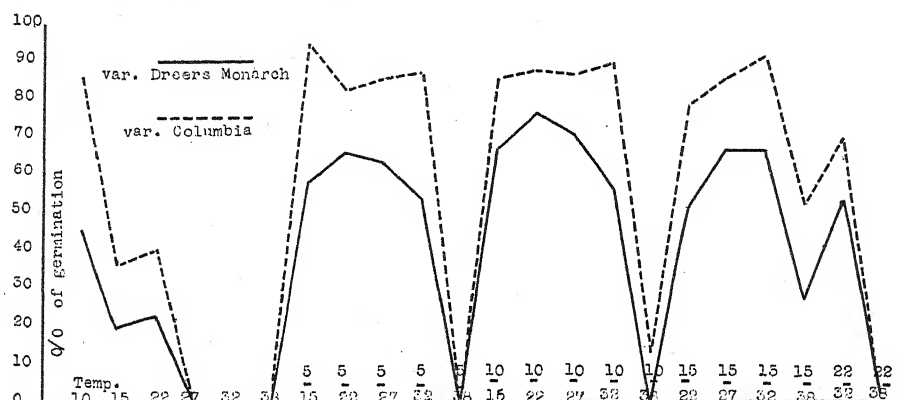
the germination of these seeds. The submerged condition is not highly favorable for the germination of blue grass seeds, probably because of low oxygen supply. Alternation of temperatures, however, has very favorable effects upon the germination of the seeds even under water.



TEXT FIG. 3. Effects of different pairs of alternating temperatures on germination of seeds of cat-tail (*Typha latifolia* L.).

*Cat-tail.* The results are shown in text figure 3. Cat-tail seeds, like Bermuda grass seeds, are decided requirers of alternate temperatures in darkness. The alternations 10°-32° C. and 15°-32° C. were favorable, while 15°-27° C. and 22°-32° C. were not. At favorable alternating temperatures cat-tail seeds germinate much better under water than they do on moist filters. This result is due, at least in part, to the fact that the water reduces the supply of oxygen to the seeds, as shown in a paper in the present issue of this JOURNAL on "The favorable effect of reduced oxygen supply upon the germination of certain seeds." Cat-tail seeds are sensitive to light, nitrogen compounds, and reduced oxygen pressures. To get best germination of intact seeds, one or more of these conditions must be combined with alternating temperatures. Rupturing of coats removes the necessity of all these conditions and gives good germination at constant temperatures in darkness, and at ordinary atmospheric oxygen concentration with distilled water.

*Celery*. The results of germination tests on moistened filter paper are shown in text figure 4. Celery responds well to a great number of alter-



TEXT FIG. 4. Effects of different pairs of alternating temperatures on germination of seeds of celery (*Apium graveolens* L.); seeds on moist filter papers.

nations. It also germinates fairly well in low constant temperatures, but not at all in higher constant temperatures. Celery seeds germinate somewhat better under water than on moist filter papers, both at low constant temperatures and at alternating temperatures.

#### Effect of Varying Duration of Exposure to each Temperature in the Alternation

Table I shows the effect produced upon total germination and upon time required for germination by varying the time the seeds were exposed to the low and high temperatures.

TABLE I. *Bermuda Grass Seeds at Daily Alternating Temperatures, 15° and 38° C.; Duplicate of 100 Seeds for each Alternation; Period of Experiment, May 22 to June 21; Glumes Intact; in Darkness*

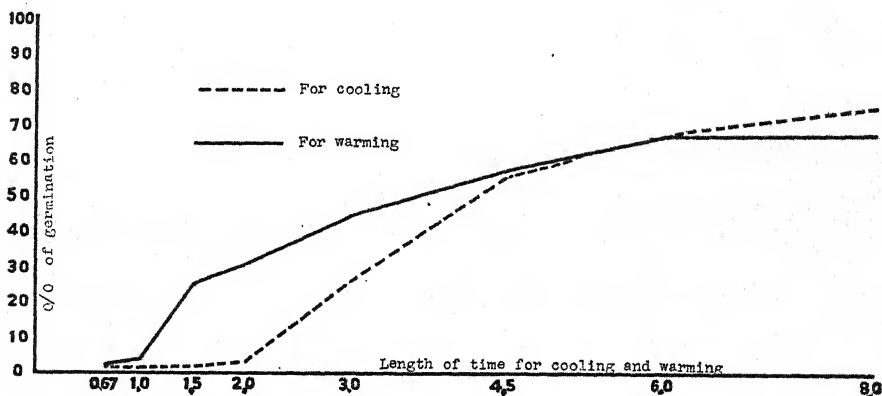
*With the Short Period at the Higher Temperature*

Length of time at higher temperature.....	40 min.	60 min.	1.5 hr.	2.0 hr.	3.0 hr.	4.5 hr.	6.0 hr.	8.0 hr.	15° const.
Percent germination after 30 days.....	2.0	3.5	25.0	30.5	44.5	58.0	67.5	68.0	0
Average number of days for germination.....	21.8	13.3	14.2	14.4	11.7	10.3	12.1	13.5	—

*With Short Period each Day at Lower Temperature*

Length of time at lower temperature.....	40 min.	60 min.	1.5 hr.	2.0 hr.	3.0 hr.	4.5 hr.	6.0 hr.	8.0 hr.	15° const.
Percent germination after 30 days.....	1.5	1.0	1.5	3.0	25.5	55.5	68.0	76.5	—
Average number of days for germination.....	11.7	4.0	9.0	10.2	11.6	12.0	11.8	11.1	—

There was little difference in the effectiveness whether the 18-hour or the 6-hour daily period was at the higher or lower temperature, the principal requirement being that the daily period of exposure to either high or low temperature must be at least 4.5–8 hours.



TEXT FIG. 5. Effects of duration of exposure to the higher and lower temperatures of the alternations; lower temperature 15° C., higher 38° C.; Bermuda grass (*Cynodon dactylon* (L.) Pers.).

Records were also kept of the germinations that took place during each exposure period in order to determine whether the germinations occurred mainly at the higher or at the lower temperature of each alternation. More germinations occurred at the high temperature than at the low, even when the length of time at the higher temperature was only one third of that at the lower temperature.

A daily period of cooling was much less effective than a daily period of warming, when the duration of the cooling or warming was less than 3 hours. A 2-hour daily period of warming gave 30.5 percent germination, while a 2-hour daily period of cooling gave only 3 percent germination.

### Effect of Nitrogen Compounds upon Germination

*Bermuda Grass.* Preliminary tests were run with duplicates of 100 seeds for each test, at 32° C. constant with 1, 0.1, 0.04, 0.02, 0.0133, and 0.01 *N* solutions of  $\text{KNO}_3$ ,  $\text{NaNO}_3$ , and  $\text{NaNO}_2$  on filter paper: Normal and one tenth normal  $\text{NaNO}_2$  retarded germination, normal and 0.04 *N*  $\text{KNO}_3$  and  $\text{NaNO}_3$  did not affect germination. All the other concentrations of these salts increased germination. After 12 days the average germination for all effective concentrations was 15.5 percent, while the checks averaged 3 percent. At the end of 12 days all seeds were transferred to the daily alternating temperature 22°–32° C., with the 6-hour period at the higher temperature. Under this condition all concentrations of the salts, except 1.0 *N*  $\text{NaNO}_2$ , gave a higher percentage of germination than the checks.



TABLE 2. *Effect of Various Nitrogen Compounds at Constant and Alternating Temperatures; Experiment Started in February and Ran for 30 Days; Germinators were Filter Paper Moistened with Distilled Water or Solutions; Duplicates of 100 Seeds in each Condition; in the Daily Alternations the 6-hour Period was at the Higher Temperature; in Darkness*

Temperature Conditions		27° C.	32° C.	38° C.	22-32° C.	22-38° C.
Cont. I (dist. water)	Percent germination after 30 days.....	0.5	0.5	0.5	55.5	72.5
	Average number of days for germination.....	4.0	4.0	4.0	8.2	7.7
Cont. II (dist. water)	Percent germination after 30 days.....	0.5	0.5	1.0	37.5	68.5
	Average number of days for germination.....	2.0	4.0	3.0	8.4	8.8
$\frac{1}{100}$ N. $\text{KNO}_3$	Percent germination after 30 days.....	0	2.0	2.0	95.0	99.0
	Average number of days for germination.....	—	3.0	2.8	4.5	4.8
$\frac{1}{100}$ N. $\text{NaNO}_3$	Percent germination after 30 days.....	0.5	0	3.5	96.0	95.0
	Average number of days for germination.....	4.0	—	4.4	4.6	4.4
$\frac{1}{100}$ N. $\text{NH}_4\text{NO}_3$	Percent germination after 30 days.....	0	0	0.5	93.0	94.5
	Average number of days for germination.....	—	—	4.0	5.4	4.6
$\frac{1}{100}$ N. $\text{HNO}_3$	Percent germination after 30 days.....	1.0	3.5	2.5	93.0	94.0
	Average number of days for germination.....	9.5	5.1	3.2	5.0	4.5
$\frac{1}{100}$ N. $\text{NaNO}_2$	Percent germination after 30 days.....	0.5	1.5	1.0	88.5	93.5
	Average number of days for germination.....	12.0	2.0	2.0	5.9	4.7

After 30 days all lots that were at constant temperatures, and hence showed poor germination, were subjected to the alternation 22°-38° C. Nitrate-treated seeds germinated over 90 percent within 6 days (the seeds that had been kept at 38° C. germinating more quickly than the seeds that had been kept at 27° C.).

It is evident that nitrate and nitrite in combination with the rather unfavorable alternations 22°-32° C. and 22°-38° C. are very effective in increasing both the rate and the percentage of germination of Bermuda grass seeds.

*Cat-tail.* Nitrates cause a marked increase in the germination of cat-tail seeds under water (reduced oxygen pressure) at the constant temperature 27° C., but they do not increase the percentage of germination of these seeds on filter papers (full oxygen pressure of the atmosphere) at the same temperature.

### Effect of Combination of Alternating Temperatures, $\text{KNO}_3$ , and Light upon Germination

*Bermuda Grass.* Seeds were first kept for 30 days at constant temperature (room temperature), some lots in darkness and some in light. In each case duplicates of 100 seeds were run in 100-cc. flasks containing 25 cc. of water or of  $\frac{N}{100}$   $\text{KNO}_3$  solution. Not more than 1.5 percent germination was obtained in any case. Similar lots treated in the same way, except that they were alternated between room temperature for 10 hours and 38° C. for 14 hours, gave 97.5 percent germination in light and 41.5 percent in darkness.

The seeds that had failed to germinate at constant temperature in light, in darkness, in water, or in  $\frac{N}{100}$   $\text{KNO}_3$  were then alternated between room temperature and 38° C. In 3 days the germination was as follows: in water + light, 52 percent; in water + darkness, 7.5 percent; in nitrate + light, 90.5 percent; in nitrate + darkness, 42 percent. After 18 days the germination in the same order was 80.5 percent, 92.5 percent, 96+ percent, 96+ percent.

The favorable effects of N compounds and light on the germination are clear. They increase both the speed and the percentage of germination. They are not as highly effective for germination, however, as are alternating temperatures. They function mainly in combination with alternating temperatures.

*Cat-tail.* Germination was tested under the following sets of conditions: in darkness at the constant temperatures of 22°, 27°, and 32° C.; in darkness at the alternating temperatures 22°–32° C.; at room temperature in light and in darkness; one series of all of the above mentioned placed in Petri dishes on moist filter paper, and a duplicate in 100-cc. Erlenmeyer flasks, with 25 cc. of water. In each case duplicates of 100 seeds were used.

At the end of 18 days germination did not exceed 2 percent at any constant temperature in darkness either in Petri dishes or in water; it was 7 percent at alternating temperatures in Petri dishes, but amounted to 88.5 percent at alternating temperatures in water; at room temperature in light the germination was 29.5 percent in Petri dishes, 76 percent in water; but in darkness not more than 1 percent germinated in either Petri dishes or water.

After 18 days all seeds kept at 22°, 27°, and 32° C. were subjected to

the alternation between 22° and 32° C. for 18 days in darkness. They gave 70 to 83 percent germination in water, but less than 3 percent in Petri dishes. After 18 days the flasks and dishes were transferred to the greenhouse with exposure to direct rays of the sun, where the daily temperature change was considerable. The seeds which did not germinate in the first and the second conditions began to germinate and gave a final germination of over 80 percent in the Petri dishes. The seeds kept in the dark by the window were subjected to light after 18 days. They started to germinate in this new condition, but did not exceed 9 percent even in water. They were finally put for another 18 days in the greenhouse, where they gave 81.5 percent additional germination in water, and 22 percent in the Petri dishes.

The effect of nitrate upon the germination of cat-tail seeds was as follows: In Petri dishes with filter paper moistened with nitrate solutions, germination was no better than on filter paper moistened with water; but when the seeds were immersed in the nitrate solution, the results after 18 days were, for  $\frac{N}{50}$  KNO<sub>3</sub>, 23.5 percent; for  $\frac{N}{100}$  KNO<sub>3</sub>, 59.0 percent; for distilled water, 1.5 percent, at 27° C. in darkness.

*Canada Blue Grass.* The results with this species are shown in table 3.

TABLE 3. *Effect of Alternating Temperatures, Light, and KNO<sub>3</sub> upon Germination of Canada Blue Grass Seed on Moist Filters in Petri Dishes*

Treatment	Percent Germination after 21 Days	
	At Constant Temperature 30° C.	At Alternating Temperature 22°-30° C.
Light + H <sub>2</sub> O.....	30.5	72.0
Darkness + H <sub>2</sub> O.....	5.0	38.5
Light + $\frac{N}{50}$ KNO <sub>3</sub> .....	60.5	95.0
Darkness + $\frac{N}{50}$ KNO <sub>3</sub> .....	21.5	83.0
Light + $\frac{N}{100}$ KNO <sub>3</sub> .....	68.0	94.5
Darkness + $\frac{N}{100}$ KNO <sub>3</sub> .....	11.5	81.0

These results show that light and nitrate are more effective for the germination of Canada blue grass than for Bermuda grass seeds. Thus, over 60 percent of the seeds germinated at constant temperature in light with nitrate. None of the three factors alone is highly effective in bringing about good germination of the seeds. To get maximum effect, the three factors must be used in combination.

#### Effect of Treatment with Concentrated Sulfuric Acid upon Germination of Bermuda Grass and Cat-tail Seeds

*Bermuda Grass.* Seeds of Bermuda grass with glumes removed were treated with concentrated sulfuric acid for 1.5, 3, 4, 5, 6, 7.5, and 9 minutes.

After thorough washing they were put to germinate on moist filter papers at 27° C. in darkness. Within 9 days seeds treated longer than 3 minutes gave 70 to 80 percent germination, while those treated 1.5 minutes gave 45 percent germination and the checks gave 1 percent. Bermuda grass seeds that had been previously soaked for 5 days had a portion of the endosperm cut away at the distal end of the embryo. These gave 86.6 percent germination in 9 days at 27° C. in darkness. Similar results were obtained with seeds that were not previously soaked. A partial removal of the coats of Bermuda grass obviates the necessity of light and alternating temperatures for germination.

*Cat-tail.* The effect of treatment with concentrated sulfuric acid is shown in table 4.

TABLE 4. *Tested at 27° C. in Darkness; Duplicates of 100 Seeds for each Condition*

	Time	Percent Germination after Following Length of Treatment				
		20 sec.	40 sec.	60 sec.	80 sec.	0 sec.
Percentage germinating on wet filter paper in Petri dishes.....	6 days	0.5	32.0	7.0	1.0	1.0
	12 "	0.5	38.0	7.0	2.5	1.0
	18 "	1.5	43.0	7.5	2.5	2.0
	24 "	1.5	44.0	7.5	2.5	2.0
Percentage germinating under water.....	6 days	0.5	45.5	9.5	2.5	3.0
	12 "	0.5	62.0	10.0	2.5	2.0
	18 "	10.0	68.5	10.0	2.5	2.5
	24 "	10.0	68.5	10.5	2.5	2.5

At the close of this experiment the seeds in the flask were transferred to the greenhouse. Within 2 weeks the seeds treated 20 seconds gave 90 percent and the checks 93.5 percent additional germination. The seeds treated 40, 60, and 80 seconds gave 3, 1, and 0.5 percent, respectively, additional germination. A partial removal of the seed coats of these seeds by sulfuric acid treatment increases greatly the germination at a constant temperature, but there is a narrow margin between the treatment that partially removes the coat effects and that which injures the seed.

#### Effect of Mechanical Opening of Seed Coat upon Germination of Cat-tail

Cat-tail seeds are very small, and in shape resemble a slender cone. The large end of the cone is covered with an inset cap with a little knob at the center. When germination occurs, the embryo pushes the cap off and continues to elongate at that end. With some practice under a dissecting microscope it is possible to remove the cap by holding the seed with one needle and gently pressing on the cap with another needle. The results of germinations with coats thus broken are given in table 5.

TABLE 5. *Effect of Breaking Seed Coat upon Germination of Cat-tail Seeds; 25 Seeds in each Condition; Darkness*

	Time	Percent Germination at the Following Temperatures						
		5°	10°	15°	22°	27°	32°	38°
On filter paper in Petri dishes . . .	5 days	Starting	Starting	96	96	96	88	100
Under water . . . . .	5 days	Starting	Starting	100	96	80	84	96

At 38° C. there was little growth following germination. At 10° C. after 12 days there was 88 and 96 percent germination in Petri dishes and flasks respectively. At 5° C. no germination occurred in 12 days. At 15°, 22°, and 32° C. rapid growth followed germination.

The need for light, reduced oxygen pressures, alternating temperatures, and nitrate solutions for the germination of cat-tail seeds disappears when the coats are broken. These needs are apparently imposed by the seed coats. Whether these conditions modify the coats and make them easier to break, or whether they act upon the embryos, giving them greater expanding pressure for breaking the coats, has not yet been determined.

### Effect of Alternating Temperatures upon the Germination of *Berberis* Seeds

Three different collections of *Berberis Thunbergii* (numbered I, II, and III), and one collection of *B. vulgaris* seeds were tested at various constant and alternating temperatures. These tests were made in Petri dishes on moist filter paper. Germination counts were made at the end of 51 days except in the case of *B. Thunbergii* III, which was counted after 40 days. The results are shown in table 6.

TABLE 6. *Effect of Alternating Temperatures on Germination of Berberis Seeds; on Moist Filters; 100 Seeds per Culture*

Percent Germination									
At Constant Temperatures					At Alternating Temperatures				
Temperature	<i>B. Thunbergii</i>			<i>B. vulgaris</i>	Temperature	<i>B. Thunbergii</i>			<i>B. vulgaris</i>
	I	II	III			I	II	III	
5° C.	64	64	3	0	10°-22° C.	100	100	92	84
10° C.	4	40	6	16	15°-32° C.	80	92		68
15° C.	0	0	1	0	22°-32° C.	24	44	3	36
22° C.	0	0	0	16	22°-38° C.	4	0		4
27° C.	0	0	0	4	10°-38° C.	16	8		0
32° C.	0	0	0	0	5°-10° C.	52	68		24
					5°-15° C.	96	84	62-	88
					5°-22° C.	100	96	83	72
					5°-32° C.	44	56	26-	52
					0°-22° C.	0	0		12

### Effect of Low-temperature Storage upon the Later Germination of *Berberis* at Constant and Alternating Temperatures

Seeds of *Berberis* were stored on moist filters from March 10 to April 11 at laboratory temperature and at temperatures of 0°, 5°, 10°, and 15° C. for the purpose of after-ripening previous to germination tests. They were then placed in germinators kept at the constant temperatures of 10°, 15°, 18° C., and at an alternating temperature (10° C. for 18 hours and 22° C. for 6 hours). The effect of this treatment upon germination after 40 days is shown in table 7.

TABLE 7. *Effect of Low-temperature Storage upon Subsequent Germination of Berberis at Constant and at Alternating Temperatures; on Moist Blotters; 100 Seeds per Culture*

Previous Moist Storage Temperature for 32 Days	Germination Temperature	Percent Germination		
		<i>B. Thun.</i> I	<i>B. Thun.</i> II	<i>B. vulgaris</i>
0° C.	10° C.	84	92	8
	15° C.	32	28	52
	18° C.	12	12	24
	10°-22° C.	100	92	76
5° C.	10° C.	96	96	40
	15° C.	68	60	92
	18° C.	84	44	72
	10°-22° C.	100	92	92
10° C.	10° C.	16	36	16
	15° C.	0	4	52
	18° C.	0	0	52
	10°-22° C.	96	96	88
15° C.	10° C.	0	0	0
	15° C.	0	0	20
	18° C.	0	0	8
	10°-22° C.	—	—	—
Room	10° C.	60	—	28
	15° C.	0	0	64
	18° C.	0	—	36
	10°-22° C.	96	92	80

The data in table 7 show that (a) alternate temperatures (10-22° C.) favored germination; (b) 32 days of storage on moist filter paper at 0° C. or at 5° C. was markedly more favorable for subsequent germination than storage in similar conditions at 10°, 15° C., or room temperature; (c) storage at 5° C. gave better results than storage at 0° C.; this was particularly true with *B. vulgaris*; (d) storage at 10° C. gave much lower subsequent germination than storage at 5° C. in the case of *B. Thunbergii*, but the *B. vulgaris* results were nearly as good at 10° C. storage as at 5° C.; (e) although room-temperature storage was much less favorable for germination than storage at 0° and 5° C., room-temperature-stored seeds could be caused to germinate almost as well as low-temperature-stored seeds provided they

were germinated at alternating temperatures ( $10^{\circ}$ – $22^{\circ}$  C.); (f) of the constant temperatures (for germination, not for storage)  $10^{\circ}$  C. was best for *B. Thunbergii* and  $15^{\circ}$  C. for *B. vulgaris*.

### Effect of Alternating Temperatures upon Germination of Low-temperature-stored Berberis Seeds

Since storage at  $5^{\circ}$  C. for 40 days was shown to favor the later germination of Berberis seeds, a test was run to determine more closely the favorable pair of alternating temperatures for the germination of Berberis seeds that had been partially after-ripened by storage at  $5^{\circ}$  C. for 40 days. The results are shown in table 8.

TABLE 8.

Temperature	Percent Germination Previous Treat- ment 40 Days at $5^{\circ}$ C. (in Sand)	No Treat- ment
$5^{\circ}$ C.....	75	1
$10^{\circ}$ C.....	75	31
$15^{\circ}$ C.....	33	0
$22^{\circ}$ C.....	13	0
$27^{\circ}$ C.....	11	0
$32^{\circ}$ C.....	10	0
$5^{\circ}$ – $15^{\circ}$ C.....	90	46
$5^{\circ}$ – $22^{\circ}$ C.....	72	28
$5^{\circ}$ – $32^{\circ}$ C.....	36	0
$10^{\circ}$ – $15^{\circ}$ C.....	91	75
$10^{\circ}$ – $22^{\circ}$ C.....	93	90
$10^{\circ}$ – $32^{\circ}$ C.....	84	80
$15^{\circ}$ – $22^{\circ}$ C.....	94	60
$15^{\circ}$ – $32^{\circ}$ C.....	83	87
$22^{\circ}$ – $32^{\circ}$ C.....	12	0

The data in table 8 show that (a) the alternating temperature pairs  $5^{\circ}$ – $15^{\circ}$ ,  $10^{\circ}$ – $15^{\circ}$ ,  $10^{\circ}$ – $22^{\circ}$ ,  $15^{\circ}$ – $22^{\circ}$  C. (and possibly also  $10^{\circ}$ – $32^{\circ}$ ;  $15^{\circ}$ – $32^{\circ}$  C.) were more favorable for the germination of partially after-ripened Berberis seeds than any constant temperature tried; (b) a difference of about  $10^{\circ}$  C. in the alternating pair gave better results than a temperature difference amounting to as much as  $17^{\circ}$  C.; (c) when the lower temperature of the alternating pair was as high as  $22^{\circ}$  C., the favorable effects of the alternation and of the previous low-temperature storage were nearly lost; (d) seeds stored at  $5^{\circ}$  C. germinated better than those not after-ripened at a low temperature; but seeds that were not treated germinated nearly as well as the treated seeds, provided the germination temperature was a favorable alternating temperature, especially  $10^{\circ}$ – $22^{\circ}$  C.

### DISCUSSION

#### Effect of Alternating Temperatures with Distilled Water on Filter Paper as the Germinator

The results of experiments on filter paper with Bermuda grass (*Cynodon dactylon* (L.) Pers.) at six constant and nine alternating temperatures, and

with celery (*Apium graveolens* L.) at six constant and sixteen alternating temperatures, agree in the main with Harrington's work on these seeds (7), although he worked with fewer constant and alternating temperatures.

Bermuda grass seeds give practically no germination at constant temperatures, but give a high percentage of germination at various alternations. Harrington got best germination, nearly 80 percent, at the alternations 15°–35° C. and 20°–35° C. In the work here reported, best germination, over 90 percent, was obtained at alternations 10°–38° C. and 15°–38° C., extremes not used by Harrington.

Celery seeds germinated well at low constant temperatures (86 percent at 10° C.), and there was fair germination at 5° C. The lowest temperature used by Harrington was 15° C., which in this work proved less favorable in some varieties than 10° C. With a rise in the constant temperatures in darkness, germination fell off, so that none occurred above 27° C. Many different alternations gave excellent germination. This was especially true when the lower temperatures in the alternation were 5°, 10°, or 15° C., and the higher temperature was 32° C. or less. The retarding effect of high temperatures is counteracted by alternations. At 22° C. constant, two varieties gave 23 and 40 percent germination respectively, while the same varieties gave 53 and 67 percent respectively at the daily alternation 22–32° C.

The fruit scales retard the germination of *Chloris ciliata* (6), *Avena elatior*, and *Holcus halepensis* (4). This is not true of Bermuda grass.

Vanha (9) found the daily alternation with *Poa pratensis* most effective when the daily high temperature period was short (4 hours). Bermuda grass seeds respond equally well to daily alternations with the high-temperature periods ranging anywhere from 6 to 18 hours. If in the daily alternations the daily period at the low or high temperature is 4 hours or less, the high temperature for the short period is more effective than the low temperature for the short period.

In the daily alternations with Bermuda grass seeds, more seeds germinate during the high-temperature periods than during the low-temperature periods, although the former are only one-third as long. Bermuda grass seeds endure higher germination temperatures than celery and some other seeds studied.

The behavior of Canada blue grass (*Poa compressa* L.) seeds at constant and alternate temperatures is very similar to that found by Harrington for *Poa pratensis* seeds. There is some germination (10 percent or more) at favorable constant temperatures. The germination at favorable alternations runs from 60 to 80 percent.

Cat-tail seeds, like Bermuda grass seeds, germinate less than 1 percent at constant temperatures, but give considerable germination, as high as 70 percent, at favorable alternations.

*Berberis Thunbergii* and *B. vulgaris* seeds respond to various alternating



temperatures. The alternation  $10^{\circ}$ – $22^{\circ}$  C. is especially favorable. These seeds also germinate at low constant temperatures,  $10^{\circ}$ ,  $15^{\circ}$ , and  $20^{\circ}$  C., if they are first after-ripened in a germinator at  $5^{\circ}$  C. This after-ripening also improves the germination at various alternations. The germination at  $5^{\circ}$  C. constant after 2 to 4 months is preceded by after-ripening at this temperature. These seeds responding to after-ripening in a germinator at a low temperature represent a class of seeds needing alternating temperatures that have not been previously mentioned in the literature.

### Effect of Alternating Temperatures upon Germination of Seeds under Water

Kinzel (8) has studied the effect of light upon the germination of seeds under water, but so far as the writer knows no work has been done upon the effect of alternating temperatures upon the germination of seeds under water.

For all the seeds studied, Bermuda grass, Canada blue grass, cat-tail, and celery, alternating temperatures, compared with constant temperatures, favor germination when the seeds are under water much as they do when the seeds are on filter paper moistened with water.

Bermuda grass seeds at the most favorable alternations germinate somewhat better under water than on filter paper moistened with water, but they show somewhat poorer germination at unfavorable alternations under water than on filter paper. The average for all alternations is about equal under water and on moist filter papers.

At alternating temperatures, Canada blue grass seed germinates much better on moist filter paper than in water; cat-tail seeds germinate much better in water than on moist filter paper; and celery germinates about equally well in both conditions. Water favors the germination of the cat-tail seeds by reducing the oxygen supply to the seeds.

### Effect of Light and N Compounds upon Germination of Seeds Favored by Alternating Temperatures

The effect of light upon the germination of seeds has been studied by many workers, and has been considered as having close relation to the temperature effects. A review of the literature on the effect of light on germination appears in a recent article by Gardner (4). Gassner (6) and others have shown the effectiveness of N compounds for forcing germination.

Nitrates and nitrites have little effect upon the germination of Bermuda grass seeds at constant temperatures, but they increase the germination at unfavorable alternations both under the solution and on filter paper moistened with the solution. Light was also effective on this seed when acting in combination with poor alternations, but not in favorable alternations. The results obtained with Bermuda grass seeds are similar to those obtained by Gassner (6) for *Ranunculus sceleratus* seeds; in both

cases alternation of temperatures is the highly effective factor, and light and N compounds supplement the effects of alternating temperatures.

The effects of light and nitrogen compounds are much more striking on the seeds of Canada blue grass; they favor the germination of these seeds even at constant temperatures and in both the favorable and the unfavorable alternations. They have a primary effect here, as well as supplementing the effect of alternating temperatures. Nitrates and light both greatly favor the germination of cat-tail seeds even at constant temperatures. Cat-tail seeds kept in a germinator in darkness for a while germinate more poorly when given favorable conditions. As in the case of *Ranunculus* seeds, they take on a darkness rigor.

### Breaking of Seed Coats in Relation to the Effect of Alternating Temperatures

It has been shown that seed coats interfere with germination in various ways; by exclusion of water, in "hard seeds"; by curtailing water-absorption or restricting swelling, in *Alisma* (3); by restricting oxygen supply to the embryos (2); by interfering with carbon dioxide elimination; and perhaps by other means not yet worked out, such as retaining inhibiting agents within the seeds.

Bryan (1) has shown that treating Bermuda grass seeds with concentrated sulfuric acid increases the germination. In a previous paragraph it is shown that both this treatment and mechanical removal of a portion of the fruit coats remove the necessity of alternating temperatures. In the same section it is shown that both sulfuric acid treatment and breaking the coats of cat-tail seeds remove the need of light, reduced oxygen pressures, alternating temperatures, or nitrate solutions for the germination of these seeds. Harrington (7) has shown that Johnson grass seeds, which respond well to alternating temperatures but germinate poorly at constant temperatures, germinate perfectly at various constant temperatures if the coats are broken.

It is not safe to assume, however, that in any of these cases the alternating temperatures and other effective conditions act solely upon the seed coats. Take the simplest assumption, namely, that the expanding force of the embryo or other seed contents is not sufficient to break the coats. The alternating temperatures, or other effective conditions, may either weaken the coats or in some way increase the expanding force of the embryo by modifying the swelling or the growth of the embryo. The coats may impose the need of alternating temperatures in various other ways even in this class of seeds. In other seeds, such as blue grass and celery, breaking the coats does not dispose of the need of alternating temperatures or of other substituting factors.

The little evidence we have indicates that in both classes of seeds mentioned above alternating temperatures have their effects on the embryos.

In an unpublished work reported December, 1924, before the Physiological Section of the Botanical Society of America, W. E. Davis has found that celery seeds show a much greater total rise in catalase activity at alternating temperatures previous to germination than is ever shown at corresponding constant temperatures. He assumes that alternating temperatures as such are modifying the growth behavior of the embryos themselves. The writer has found the same for Bermuda grass seeds, where, in contrast to celery, the coats impose the necessity of alternating temperatures. Upon the whole it seems safe to say, however, that we still have to learn in the main the mechanism, or perhaps the various sorts of mechanisms, by which alternating temperatures and their substituting factors promote germination.

#### SUMMARY

1. Alternating temperatures were effective in germination of seeds of *Cynodon dactylon*, *Poa compressa*, *Typha latifolia*, *Apium graveolens*, and *Berberis Thunbergii*.

2. More seeds of *Cynodon dactylon* started to germinate at the higher temperature of the alternation than at the lower, even though the length of time at the former temperature was only one third that at the latter.

3. Alternating temperatures were necessary and sufficient to germinate intact seeds of *Cynodon dactylon* and *Typha latifolia*. *Poa compressa* seeds germinated about 10 percent at various constant temperatures, and did not give the highest percentage of germination even under the best alternating temperatures.

4. *Apium graveolens* seeds germinated as well at the low temperature (10° C.) as at alternating temperatures. *Berberis Thunbergii* seeds also germinated at low (5° C.) or alternating temperatures. After keeping the latter for 30 days at 5° C., they were able to germinate at higher constant temperatures.

5. *Cynodon dactylon* seeds germinate slightly better in water than on filter paper, and *Poa compressa* seeds germinate slightly less in water than on filter paper when they are under favorable alternating temperatures. *Apium graveolens* seeds germinate to as high percentage in water as on filter paper. *Typha latifolia* seeds germinate much better in water than on filter paper.

6. Light and nitrate as well as nitrite were effective in the germination of *Cynodon* seeds, when applied together with alternating temperatures. The effects of light and nitrate were more striking on *Poa* seeds. They increased the percentage of germination at constant and alternating temperatures. The highest percentage of germination of *Poa* seeds was obtained when these three factors were used in combination.

7. Nitrate did not affect the germination of celery seeds, and light was effective in germination only when unfavorably high temperatures were used.

8. Mechanical treatment or treatment with  $H_2SO_4$  (concentrated) was effective in forcing the germination of *Cynodon* and *Typha* seeds at constant temperatures.

9. *Cynodon dactylon*, *Poa compressa*, and *Typha latifolia* seeds germinated in boiled distilled water covered with paraffin oil; but *Cynodon dactylon* seeds and *Typha latifolia* seeds, coats entire or broken, did not germinate in a vacuum.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.,  
YONKERS, NEW YORK

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## THE FAVORABLE EFFECT OF REDUCED OXYGEN SUPPLY UPON THE GERMINATION OF CERTAIN SEEDS

TOSHITARO MORINAGA

(Received for publication December 4, 1925)<sup>1</sup>

### INTRODUCTION

A number of workers (1, 2, 3, 5) have shown that *increasing* the partial oxygen pressure of the atmosphere brings about or improves the germination of intact seeds of several species of plants. Work reported in this paper, however, shows that *reducing* the oxygen pressure has a very beneficial effect upon the germination of intact cat-tail seeds at a variety of temperatures, and a marked effect upon the germination of Bermuda grass seeds.

With both cat-tail and Bermuda grass seeds, diluting the air with nitrogen or hydrogen favored germination. The optimum for cat-tail seeds was obtained by diluting the air with 40 to 80 percent of these gases, and for Bermuda grass with 40 to 60 percent.

Cat-tail seeds germinated much better in atmospheres of one hundredth normal oxygen pressure than in normal oxygen pressure, but chlorophyll-development in the seedlings required greater pressure than this, namely, about 4 percent normal oxygen pressure.

These results must not be taken to mean that reduced oxygen pressure will favor the germination of most seeds. It is probable that relatively few seeds are thus favored in germination.

### EXPERIMENTAL RESULTS

#### Effect of Reduced Oxygen Pressures on Germination of Seeds of Cat-tail (*Typha latifolia* L.)

To secure the oxygen pressures desired, Petri dishes without covers, containing the seeds on moist filter papers, were placed on a tripod in a pan. Battery jars were inverted over the Petri dishes and tripods. Water was poured in the pans to give water seals, as well as water to displace the air to be withdrawn. Sufficient air to give the desired mixture was then withdrawn and replaced by hydrogen or nitrogen. All lots were then placed in the greenhouse at temperatures with a daily range from about 15° to about 30° C. The results obtained when hydrogen was mixed with air are shown in table 1 and in text figure 1.

<sup>1</sup> Published, at the expense of the Boyce Thompson Institute for Plant Research, out of the order determined by the date of receipt of the manuscript.

TABLE 1. *Effect of Reduced Oxygen Pressure on Germination of Cat-tail Seeds; Air Diluted with Hydrogen Gas; Duplicates of 200 Seeds for each Condition*

Time	Percent Germination						
	Check in Petri Dish*	Check under Sealed Jar†	20% H <sub>2</sub> , 80% Air	40% H <sub>2</sub> , 60% Air	60% H <sub>2</sub> , 40% Air	80% H <sub>2</sub> , 20% Air	90% H <sub>2</sub> , 10% Air
2 days.....	0	1.3	88.0	96.8	95.8	95.5	93.5
4 days.....	0	42.3	94.8	97.5	96.5	97.3	96.3
6 days.....	0	80.8					
8 days.....	1.3	85.5					
10 days.....	3.8	86.0					

\* Seeds in Petri dishes, loosely covered, exposed to air.

† Seeds in air inside inverted battery jars with water seal.

The results obtained when air was replaced by nitrogen instead of hydrogen are shown in table 2 and text figure 2.

TABLE 2. *Effect of Reduced Oxygen Pressure on Germination of Cat-tail Seeds; Air Diluted with Nitrogen Gas; Duplicates of 200 Seeds for each Condition*

Time	Percent Germination						
	Check in Petri Dish*	Check under Sealed Jar†	20% N <sub>2</sub> , 80% Air	40% N <sub>2</sub> , 60% Air	60% N <sub>2</sub> , 40% Air	80% N <sub>2</sub> , 20% Air	90% N <sub>2</sub> , 10% Air
2 days.....	0	0	10.3	49.0	95.5	94.3	89.8
4 days.....	0	42.5	93.8	94.5	97.8	96.3	97.8
6 days.....	0	70.5					
8 days.....	1.3	83.5					
10 days.....	3.8	84.5					

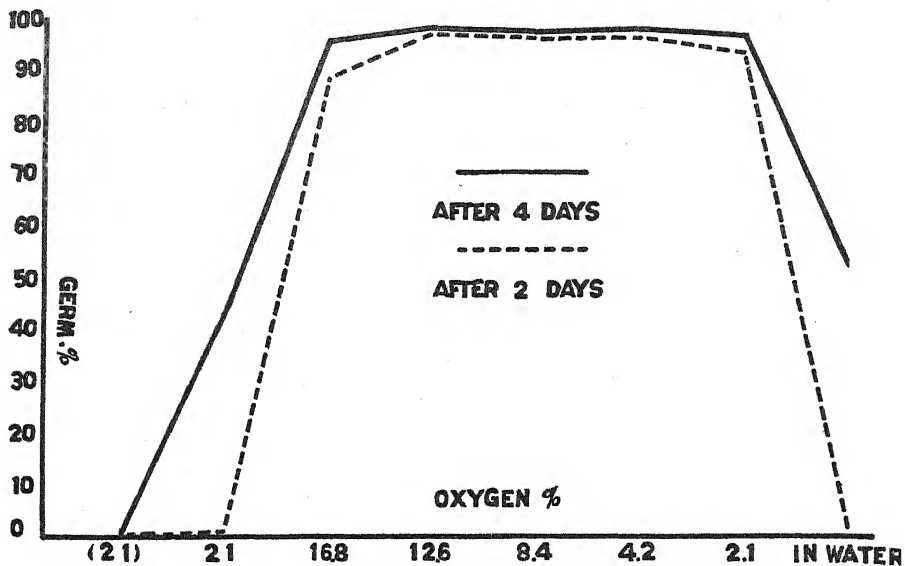
\* Seeds in Petri dishes, loosely covered, exposed to air.

† Seeds in air inside inverted battery jars with water seal.

The results obtained by diluting air with nitrogen were similar to those obtained by diluting with hydrogen. From tables 1 and 2 it is evident that prompt and almost complete germination was obtained with cat-tail seeds on moist filter paper when the partial oxygen pressure of the atmosphere was reduced by additions of hydrogen or nitrogen. Favorable effects appeared with any additions from 20 to 90 percent, the most favorable being with additions of 40 to 80 percent.

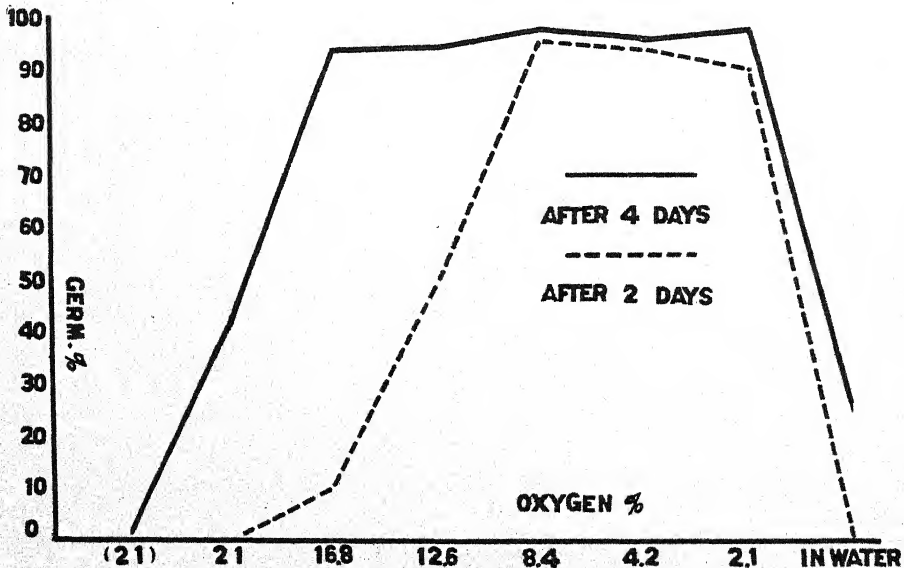
From tables 1 and 2 it can be seen that, in the check lots in Petri dishes loosely covered with easy access to air, the germination did not exceed 4 percent; in the check lot that was placed in air under an inverted battery jar protected from the outside air by a water seal, germination proceeded slowly and finally reached about 85 percent.

Further evidence regarding the effect of sealing the germination chambers was obtained from the following experiment. Two hundred seeds each



TEXT FIG. 1. Effect of reduced oxygen pressure on germination of cat-tail seeds; air diluted with hydrogen gas.

were put on wet filter paper in large Petri dishes, and one set of these dishes was sealed with modeling clay with a little dish of NaOH solution inside; a second set was arranged similarly without the caustic solution and a third set was placed in unsealed Petri dishes. After 10 days in the



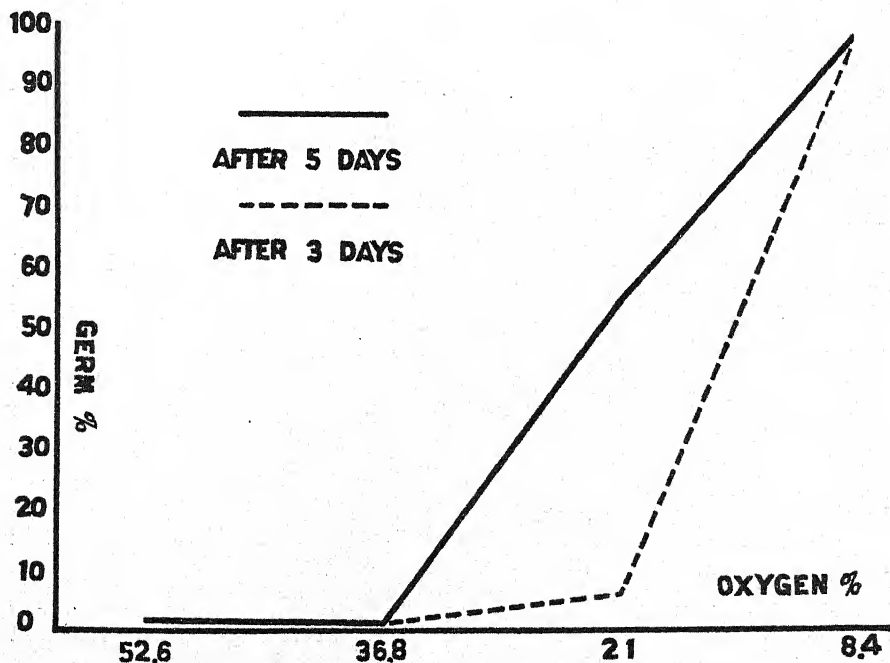
TEXT FIG. 2. Effect of reduced oxygen pressure on germination of cat-tail seeds; air diluted with nitrogen gas.

greenhouse 85.5 percent of seeds germinated in the dishes with NaOH, 94.8 percent in the dishes without, and only 2.8 percent of seeds germinated in the dishes that were not sealed.

The reasons for better germination inside the battery jars than in Petri dishes are not known. That this result is not due to a reduction in the oxygen concentration by the respiration of the seeds is indicated by the fact that the oxygen concentration inside the closed battery jar after 8 days had been reduced only from 20.4 percent to 19.4 percent. It may be that a more favorable alternation of temperature was obtained inside the battery jar. Further work on this point needs to be carried out.

#### Effect of Increased and Decreased Oxygen Pressures on Germination of Cat-tail Seeds

In order to obtain further evidence on the rôle of oxygen in the germination of cat-tail seeds, increased oxygen concentrations, obtained by adding oxygen to air, were compared with decreased oxygen concentrations, obtained by adding nitrogen to air. The results are shown in table 3 and text figure 3. These experiments were performed under battery jars as were the previous ones.



TEXT FIG. 3. Effects of increased and decreased oxygen concentrations upon germination of cat-tail seeds.



TABLE 3. *Comparison of Effects of Increased and Decreased Oxygen Concentrations on the Germination of Cat-tail Seeds; Duplicates of 200 Seeds for each Condition*

Time	Percent Germination			
	40% O <sub>2</sub> Added to Air	20% O <sub>2</sub> Added to Air	Air	60% N <sub>2</sub> Added to Air
3 days.....	1.8	1.3	6.3	97.3
5 days.....	1.8	1.3	54.3	98.3
9 days.....	1.8	1.3	67.5	

Table 3 shows clearly the favorable effect of reduced oxygen concentrations on the germination of cat-tail seeds. The fact that the seeds in the air lot germinated slowly and gave 67.5 percent germination in 9 days shows that some other factor was operating in conjunction with reduced oxygen pressure in forcing germination; but the favorable effect of this factor was nullified, at least during the time of this experiment, by the addition of extra oxygen to the battery jar.

#### Effect of Lower Concentrations of Oxygen on the Germination of Cat-tail Seeds

In another set of experiments under battery jars in the greenhouse, 94 percent of the seeds germinated in 99 percent hydrogen mixture with air after 4 days, but gave white seedlings. In 96 percent hydrogen the germination was as good and the seedlings were yellowish green. Evidently the oxygen supply required for germination is less than that required for chlorophyll-development, as Crocker and Davis (4) found for *Alisma Plantago*.

Can cat-tail seeds germinate in complete absence of gaseous oxygen? The desired number of seeds was placed in a 25-cc. distilling flask with 10 cc. of water. The neck of the flask was sealed, and the side tube was drawn down to a capillary at one region. The side tube was then connected with a high vacuum pump, and the water in the flask was boiled under vacuum in a water bath at 40°-45° C. for 30 minutes, or until about 1 cc. of water remained in the flask. The side tube was then sealed off while still being evacuated and boiled. In consequence of evaporation under the vacuum, the seeds always remained far below the temperature of the bath so that there was no injury from high temperature. The flasks were then put in the most favorable conditions for germination. With cat-tail seeds this involved also breaking the seed coats before sealing in the flasks. No germination was obtained when, by this method, oxygen was removed completely or nearly completely from both water and seeds.

### Effect of Reduced Oxygen Pressures upon Germination of Bermuda Grass Seeds

The effect of reduced oxygen concentrations upon the germination of Bermuda grass (*Cynodon dactylon* (L.) Pers.) was then tested, using the same method that was employed in the experiments with cat-tail seeds. The oxygen concentration was reduced by diluting air with hydrogen. The results are shown in table 4.

TABLE 4. *Effect of Reduced Oxygen Pressures upon Germination of Bermuda Grass; Duplicates of 100 Seeds Used in each Condition*

Time	Percent Germination						
	Check in Petri Dish*	Check under Sealed Jar†	20% H <sub>2</sub> , 80% Air	40% H <sub>2</sub> , 60% Air	60% H <sub>2</sub> , 40% Air	80% H <sub>2</sub> , 20% Air	90% H <sub>2</sub> , 10% Air
6 days.....	1.0	15.5	17.0	26.0	32.0	21.5	10.5
8 days.....	15.5	62.0	73.0	81.5	83.5	75.5	53.0
10 days.....	24.5	73.5	84.5	89.0	91.0	85.0	63.5

\* Seeds in Petri dishes, loosely covered, exposed to air.

† Seeds in air inside inverted battery jars with water seal.

When this experiment was repeated with 60-percent nitrogen and 60-percent hydrogen mixtures with the air, the former gave 89.5 percent and the latter 85.5 percent germination after 10 days as against 55 percent for the checks.

The data in table 4 show: (1) That a reduction in oxygen concentration of air favored the germination of Bermuda grass, although the favorable effect was less marked than for cat-tail seeds; (2) the favorable mixtures were 40–60 percent hydrogen mixed with 60–40 percent air. With 90 percent hydrogen and 10 percent air the germination was not as good as with the checks under the sealed jar receiving no treatment.

### Influence of Seed Coat upon Oxygen Requirement for Germination of Cat-tail Seeds

In the case of cat-tail seeds, at least, the favoring effects of reduced oxygen pressures disappeared when the seed coats were broken. When this was done these seeds germinated well, at various constant temperatures, in oxygen pressures ranging anywhere from 1 to 90 percent oxygen of full atmospheric pressure. The mechanism by which reduced oxygen pressures favor the germination of intact seeds, when the naked embryos are so indifferent to broad variations in oxygen pressure, is not known. It is not safe to assume, however, that the reduced oxygen pressure affects the coats alone, although this may be the case. It may also act upon the

embryo mainly, as is suggested for alternating temperatures in a preceding paper in the present issue of this JOURNAL on the "Effect of alternating temperatures upon the germination of seeds."

### Effect of Reduced Oxygen Pressures on the Germination of Certain Other Seeds

Reduced oxygen pressure also favors the germination of water cress (*Roripa nasturtium* Rusby) and white clover (*Trifolium repens* L.) seeds at 22° C. or above, as shown by the results reported in a paper "Germination of seeds under water," also published in the present number of this JOURNAL. Reduced oxygen pressure does not favor the germination of sweet clover (*Melilotus alba* Desr.) and alfalfa (*Medicago sativa* L.) seeds at any temperature, as shown in the same paper.

#### SUMMARY

1. Seeds of cat-tail (*Typha latifolia* L.), which germinate poorly or not at all in air, germinate promptly when the oxygen concentration of the air is reduced by diluting with hydrogen or nitrogen.

2. Favorable concentrations were obtained by diluting the air with 40 to 80 percent (by volume) of hydrogen or nitrogen, approximately 96 percent germination resulting.

3. When the oxygen concentration was increased by adding 20 percent oxygen to air, germination was 1.3 percent; but adding 60 percent nitrogen to air brought about prompt germination (97-98 percent).

4. Cat-tail seeds germinated 94 percent in a 99-percent hydrogen mixture with air, but the resulting seedlings were white, there being insufficient oxygen for chlorophyll-development.

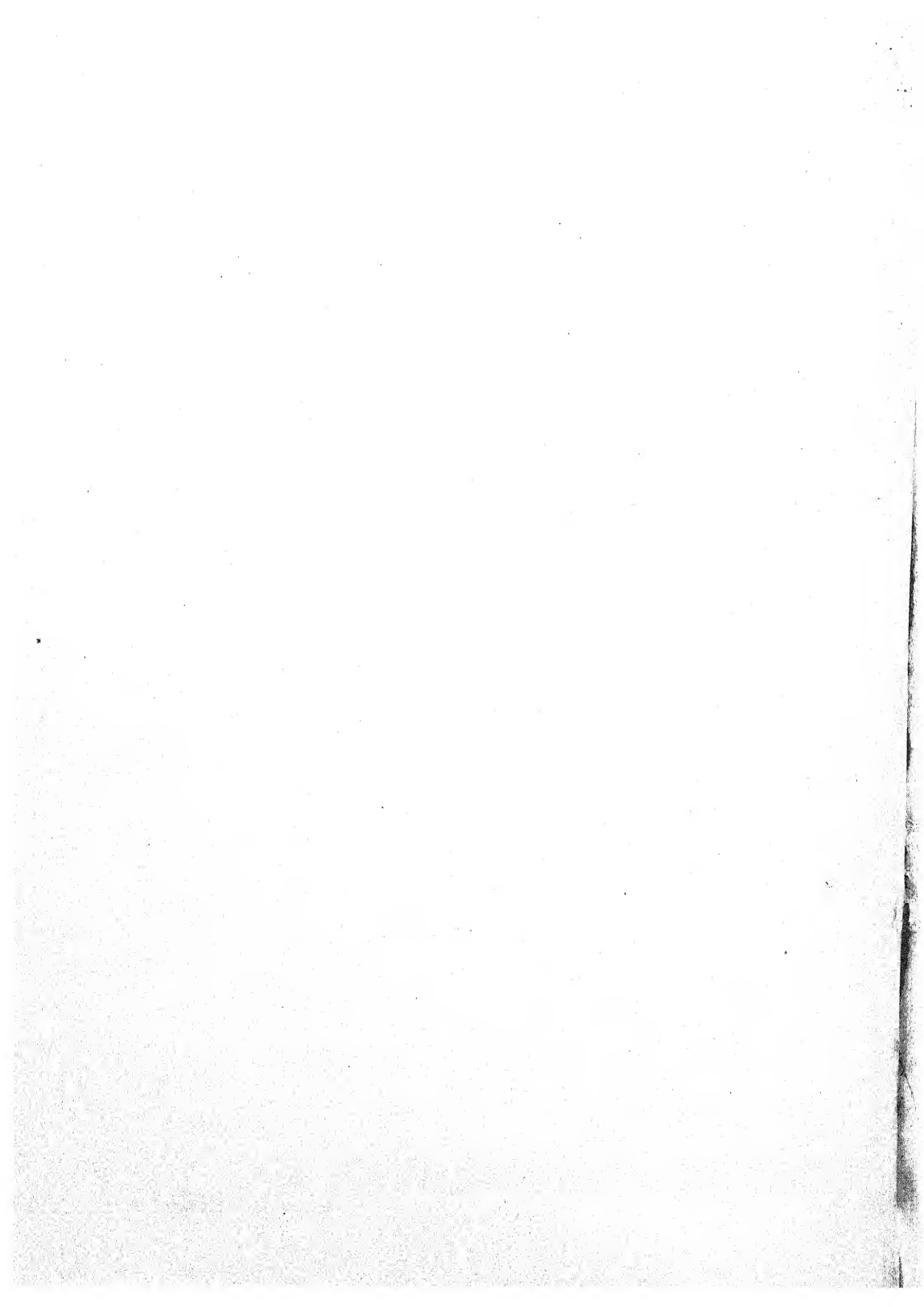
5. When special precautions were taken to remove oxygen completely or nearly completely from the flasks, liquids, and seeds, no germination resulted. A small supply of oxygen is necessary for germination.

6. The favoring effects of reduced oxygen pressures disappear when the seed coats are broken. Under this condition germination proceeds readily in oxygen pressures ranging from 1 percent to 90 percent of the full atmospheric oxygen pressure.

7. Bermuda grass (*Cynodon dactylon* (L.) Pers.) also germinates better when the oxygen pressure of the air is reduced by diluting with hydrogen or nitrogen, although the favorable effect was less marked than for cat-tail seeds; favorable mixtures were obtained by diluting air with 40-60 percent hydrogen or nitrogen.

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## AN ORGANISM OF TOMATO MOSAIC

SOPHIA H. ECKERSON

(WITH PLATES XIX-XXII)

### Introduction

THIS study was begun in 1922 at the University of Wisconsin, in connection with work on wheat rosette with H. H. MCKINNEY, while on part-time appointment from the Cereals Division of the United States Department of Agriculture. The early studies of tomato mosaic were made in 1922 and 1923, with the cooperation of E. J. KRAUS of the University of Wisconsin. Dr. KRAUS also turned over to me abundant material, both diseased and healthy plants, for the study of mosaic in *Hippeastrum Johnsoni* and pepper. The figures in pl. XIX were drawn from slides made at that time. The work has been continued at Boyce Thompson Institute. At present H. R. KRAYBILL and the writer are starting a study combining differential filtration, inoculation, and microchemical examination, which should bring out new facts about mosaic. In the meantime, I shall describe organisms seen in mosaic plants, especially the one in tomato, which has been observed most carefully.

### Motile organisms

WHEAT.—Motile organisms were first seen in the rosette wheat. In the first few days of the infection there were numerous tiny flagellated forms ( $2-4\ \mu$ ) in the cells. A week later there were fewer of these, but many larger motile forms ( $5-7\ \mu$ ). Still later the large non-motile bodies predominated. These different forms always appeared in this sequence.

TOMATO.—An examination of mottled leaves of mosaic plants available revealed motile organisms in all. The very young, not yet mottled leaves of the mosaic plants also had hundreds of tiny rapidly moving organisms (fig. 5 right) in the mesophyll cells; while in the phloem cells were elongated forms having typical flagellate movement. In the older, badly mottled leaves were many melon-seed-

shaped, sporelike forms having a hyaline membrane (fig. 8 right). These were imbedded in the few remaining chloroplasts as well as free in the cells. These sporelike forms were puzzling, since they were not stainable, either living or in smears. More is known about them now, but they are still somewhat puzzling.

VITAL STAINS.—Much time was given to observation of the living organisms within the living cells of the plant. Of the many vital stains tested for differentiation, the combination of methylene blue and eosin was the most satisfactory for the small forms. These small ovoid forms, having amoeboid movement (fig. 6), and the long thin flagellate forms stain readily. When the stains are used in very dilute solution, the organisms are tinged with the eosin, while their nuclei are blue. Recently I have used polychrome methylene blue instead of the eosin combination. Since the nuclei of these small organisms are denser than the somewhat transparent body, they can be seen clearly in living unstained condition by proper arrangement of the light.

Brilliant cresyl blue is satisfactory for the larger forms (fig. 7 left). If used in combination with acid fuchsin, the organisms are tinged with red, while their nuclei are blue. This combination was also used with good results on wheat rosette for distinguishing the big bodies from the cell nuclei. In that case the stains were made up in formalin.<sup>1</sup> The bodies were pink with blue nuclei. The cell nuclei were blue with pink nucleoles. This was excellent for diagnosis, but the colors were not permanent.

Sections of fresh tissue were stained also with iron haematoxylin and with azur-eosin under observation at the microscope. This was for localization of the organisms, and to be certain that the ones stained in the smears were those observed living in the cells.

ORGANISMS IN CHLOROPLASTS.—This year Dr. KRAYBILL and I have inoculated several series of young tomato plants with filtered juice from mosaic plants. Alternate leaflets of three or four leaves on each plant were scratched with a fine needle, and the juice brushed over them. I examined the uninoculated leaflets, opposite

<sup>1</sup> (1) 0.25 gm. brilliant cresyl blue, 90 cc. 4 per cent formalin, and 10 cc. methyl alcohol; (2) 0.05 gm. acid fuchsin, 0.05 gm. orange G, 95 cc. 4 per cent formalin, and 5 cc. 1 per cent acetic acid. Mix a few drops of (1) and (2); stain lightly (about 10 min.).—Enzlyk Mik. Tech. 1910.

those inoculated, at intervals of twenty-four hours for the first few days.

Twenty-four hours after inoculation, in leaflets opposite inoculated leaflets, there were tiny flagellated organisms (fig. 13 and fig. 15 extreme left) in the veins and in the adjacent mesophyll cells. None were found in other regions. Most of the chloroplasts of these cells were in healthy condition. Many had several small starch grains at the surface (fig. 9 left), but in a few of the cells nearest the veins the chloroplasts were beginning to show signs of dissolution. Small organisms appeared to be entering some of these plastids (fig. 9 middle), while they had entered and were beginning to swim around in little pools in others (fig. 9 right).

Three days after inoculation there were tiny flagellated forms, and also somewhat larger forms (fig. 14) in great numbers throughout the mesophyll tissue.

Five days after inoculation many cells of the mesophyll tissue were in bad condition. Their chloroplasts were no longer held by the cytoplasm in orderly arrangement, but were floating free. Most of these chloroplasts showed progressive liquefaction, while the organisms within them were larger (fig. 10 left and middle). Many of the chloroplasts were almost wholly liquefied (fig. 10 right and fig. 11 left).

Seven days after inoculation some chloroplasts of the palisade cells were in process of liquefaction. Many of the remaining plastids contained non-motile bodies which seemed to be early stages of spore formation (fig. 11 middle and right).

Ten days after inoculation some of the leaflets were beginning to show mottling. Within the leaf groups of palisade cells were partially disorganized; the cytoplasm was gone (or liquefied); and the chloroplasts were in disarray, many partially dissolved, others containing spores (fig. 12). These groups of disorganized cells were usually bounded by groups of cells apparently in perfectly healthy condition.

The foregoing description is from our latest series. The specific number of days after inoculation for any stage varied a little in the different series, but the sequence was the same in all. Moreover, the sequence in inoculated plants is the same as that from the youngest



leaf of any mosaic plant down to the fifth or sixth from the tip. The only difference found was in the later stages of the disease. In plants growing under good cultural conditions in the field, there are fewer spores but many more big slowly motile forms (fig. 16).

**SPORES.**—Twenty to thirty days after inoculation, although there were still groups of good cells, most of them had become filled with melon-seed-shaped spores. These cells were completely disorganized, without either cytoplasm or chloroplasts. They were like little boxes filled with pebbles which spilled out when the walls were cut.

The mature spores (fig. 8 right) have a highly refractive, hyaline membrane of slight permeability, and are difficult to stain. Recently, however, rather good results have been obtained by fixing in methyl alcohol, staining overnight in iron haematoxylin, and destaining.

Before the spores are mature, that is before the hyaline membrane is formed, the walls are very permeable. Their contents are difficult to stain in place, because they are shot out during fixation. Many spore cases show a spiral filament still attached. There are always dozens of detached tails on the slide, and among these many tiny flagellated forms. On one particularly good slide the tiny organisms were in such a position on the filament that it seemed that they must have been shot out together (fig. 18).

Peculiar bodies, appearing much like Japanese lanterns in the living tissue, were caught in two of the inoculated series (fig. 17 left two figs.; fig. 18 left). When stained with haematoxylin they show clearly two or three organisms within each. These tiny organisms are the same type as those present at the beginning of the disease (fig. 13 left).

#### Organisms in other mosaic plants

For comparison with tomato mosaic, several other plants were examined. I found similar organisms in all, although not necessarily the same organism. There are slight differences in form and considerable differences in size, but the general appearance and behavior are the same. There are tiny organisms in the youngest leaves, also in inoculated plants of *Hippeastrum Johnsoni* (fig. 2). There are elongated flagellate forms in the phloem tissue of older leaves (*Hip-*

*peastrum Johnsoni*, fig. 3; *Dahlia*, fig. 22 left; squash, fig. 25 left). There are also the larger slow moving forms (*H. Johnsoni*, fig. 4 left; squash, fig. 26 left). In badly mottled leaves of all there are spore forms (*Dahlia*, fig. 23 right; squash, fig. 26 right; *H. Johnsoni*, fig. 4 right).

The spores from *Dahlia* should be good material for a study of development, structure, contents, and germination of these, as yet little understood bodies. I hope to make such a study next summer.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH  
YONKERS, N.Y.

### EXPLANATION OF PLATES XIX-XXII

All figures were drawn with a Bausch & Lomb 1.8 mm. fluorite objective, 1.3 N. A., and Leitz periplane ocular 20X. All magnifications are approximately 3200 diameters.

#### PLATE XIX

FIG. 1.—Pepper, with iron-alum haematoxylin: tiny flagellated forms; elongated forms; at right, amoeboid forms.

FIGS. 2-4.—*Hippeastrum Johnsoni*, with iron-alum haematoxylin: fig. 2, from young leaf of seedling 10 days after inoculation; fig. 3, elongated flagellate forms from phloem of leaf of mature plant; fig. 4, from badly mottled leaf; at right, stages in spore formation.

FIGS. 5-8.—Tomato, with azur-eosin: fig. 5, from youngest leaf of mosaic plant, division; fig. 6, from young mottled leaf; fig. 7, three figs. at right probably stages in spore formation; fig. 8, stages in spore formation; at right three spores with hyaline membranes; a few tiny flagellated forms found on slide among these spores.

#### PLATE XX

FIGS. 9-12.—Tomato, chloroplasts from leaflets opposite inoculated leaflets; with two exceptions fixed in 1 per cent osmic, stained with iron haematoxylin 10 minutes at 45° C.: fig. 9, from mesophyll cells bordering veins, 24 hours after inoculation; left figure with small starch grains, which show clear cross in polarized light; middle figure, tiny organisms entering plastid; right figure, organisms in plastid, which is beginning to liquefy; fig. 10, from mesophyll cells 5 days after inoculation; increasing liquefaction of plastid; right figure from living unstained tissue; nuclei seen by oblique light; nucleus not visible in the denser organism; spore with hyaline membrane imbedded in remainder of plastid; fig. 11, left figure similar to right figure of fig. 10; middle and right figures from palisade cells 7 days after inoculation; fig. 12, from palisade cells 10 days after inoculation; leaflet slightly mottled; spiral filaments shot out by osmic acid.

## PLATE XXI

FIGS. 13-19.—Tomato: fig. 13, tiny flagellated forms having rapid buzzing movement, predominating in youngest leaf, also in leaf the first few days after inoculation; fig. 14, larger forms having active amoeboid movement, found in second and third leaf below tip, also in leaf third and fourth days after inoculation; fig. 15, elongated forms having typical flagellate movement, found chiefly in phloem parenchyma; fig. 16, larger less active (except when dividing) forms from fifth and sixth (from tip) mottled leaves from large plants in garden; fig. 17, two figures at left from palisade cells 5 days after inoculation; figures at right probably spore forms, from phloem cells; fig. 18, two figures at left containing tiny organisms similar to those in fig. 13; spores having spiral filaments, extruded during fixation; at extreme right tiny flagellate forms seemingly ready to start the cycle anew; fig. 19, stages in formation of large spores, found chiefly in badly mottled leaves from garden.

## PLATE XXII

FIG. 20.—Strawflower: figures at leaf from youngest leaf; at right from mottled leaf; figure at extreme right beginning spore formation (?).

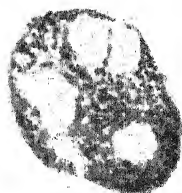
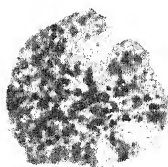
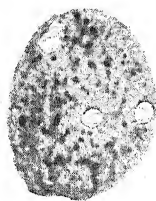
FIGS. 21-23.—Dahlia: fig. 21, from yellow tip of stunted badly diseased plant; fig. 22, from older mottled leaf; fig. 23, from large mottled leaf of vigorous shoot, stages in spore formation.

FIGS. 24-26.—Squash: fig. 24, from young leaf of badly diseased and distorted plant; fig. 25, elongated flagellate forms; fig. 26, from badly mottled leaf; at left large forms having slow movement; at right spore formation.



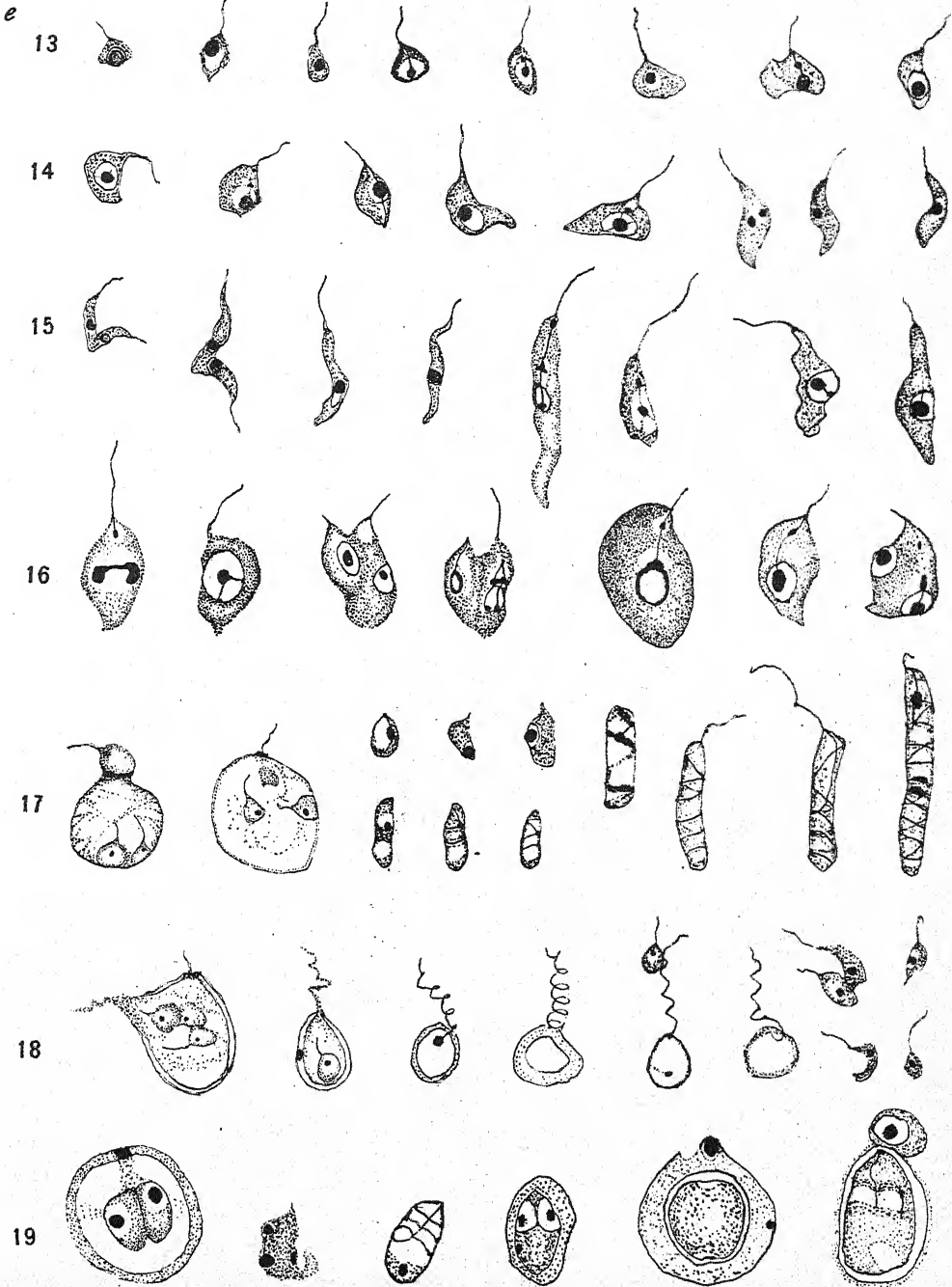
S.H.E.





ECKERSON on TOMATO MOSAIC



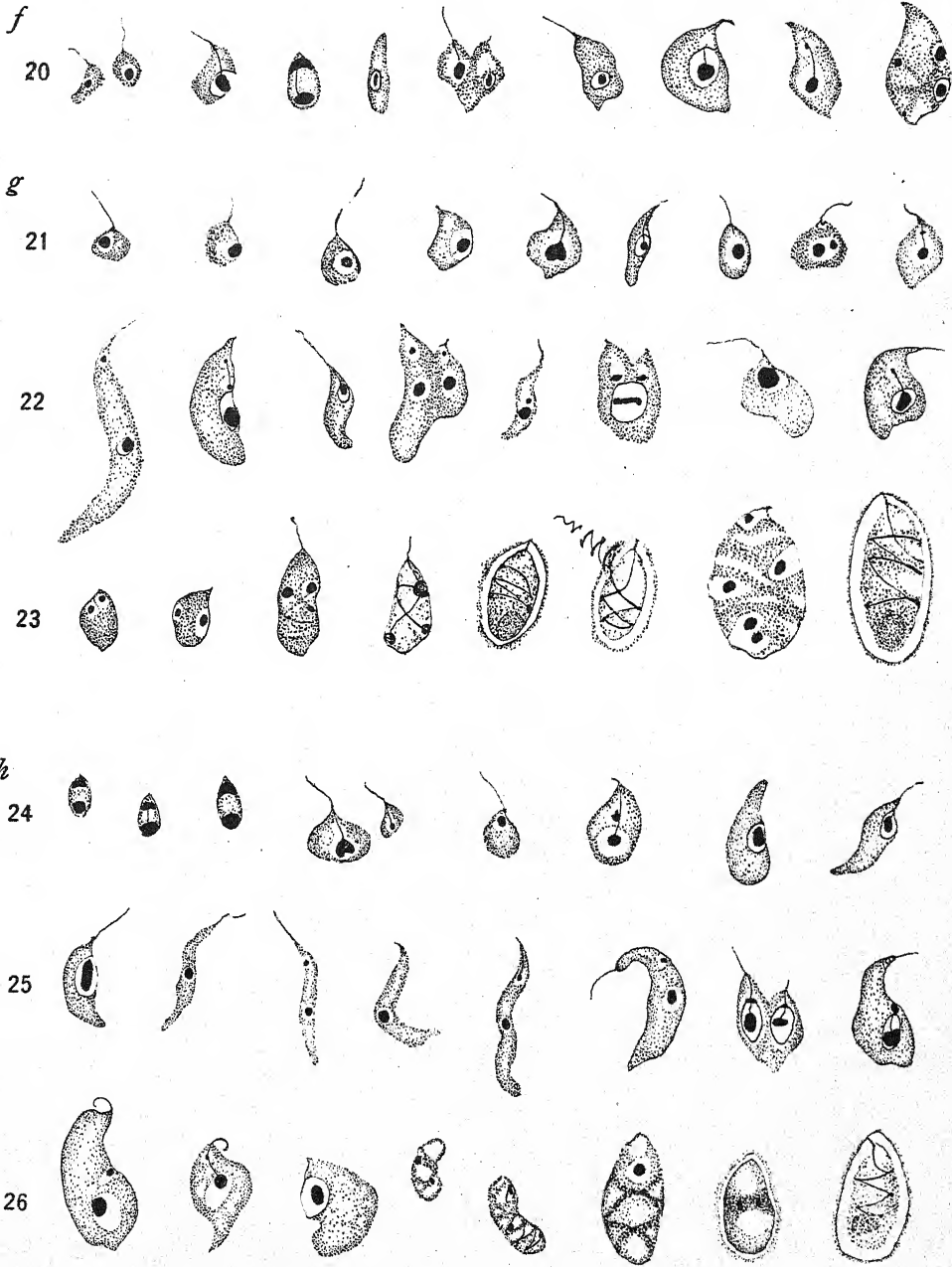


S.H.E.

ECKERSON on TOMATO MOSAIC







S.H.S.



## GROWTH OF SEEDLING IN RELATION TO COMPOSITION OF SEED

MARY E. REID

(WITH PLATES XVI-XVIII)

Experiments with seedlings varying in their proportions of carbohydrates to nitrogen have been conducted by allowing the seedlings to grow on their own nitrogen reserves to maximum size in light, in darkness, and in light in atmospheres containing 0.4 per cent  $\text{CO}_2$  and lacking  $\text{CO}_2$ ,<sup>1</sup> except for small amounts given off in respiration. Corresponding tests have also been conducted in which the seedlings have been given nitrate nitrogen. The seedlings were grown in sterilized quartz sand, kept moist by the use of nutrient solutions. The object was to study the total growth-producing value of the foods stored in the different seeds, and the relations of varying proportions of these organic foods in altering the shoot to root ratios. Of particular interest have been the relations of total nitrogen to growth, and the modifying influences of additional carbohydrates, synthesized in the light, and of nitrate nitrogen on seedlings grown in light and in darkness. All records of growth are based on measurements of size and green weights. In calculating the efficiency of the food reserves for growth, the seed coats were removed.

### 1. Seedlings grown in darkness at $21^\circ$ – $24^\circ$ C.

(a) ON THEIR OWN NITROGEN RESERVES.—The food reserves having a high proportion of nitrogen and fat have been the most efficient in producing growth in darkness. The total growth in grams of green material produced per gram of original dry food material ranged from 4.86 gm. for low-protein corn to 32.51 gm. for sunflower, a high-protein, high-oil seed. The shoot to root ratios ranged from 2.01 for low-protein wheat to 8.37 for sunflower. Similar differences between varieties occur as between species; for example, high-protein corn and high-protein wheat both have higher

<sup>1</sup> The measurements of  $\text{CO}_2$  concentration were made by Dr. WARD B. DAVIS.

shoot to root ratios than the corresponding types that are low in protein.

(b) WITH NITRATES.—Only a few of the seedlings, and these to only a small extent, responded to nitrates with an increase in total growth. Nitrates increased the growth of high-protein seedlings in as many instances as in seedlings of low-protein seeds. In nearly all cases there has been some modification of the proportions of shoots to roots, the seedlings receiving nitrates having relatively the greater weight of shoots. It seems that, although nitrates in most cases do not increase the quantity of growth in darkness, they do have the capacity for modifying the type of response.

## 2. Seedlings grown in atmospheres containing different amounts of $\text{CO}_2$

The atmospheres used contained high concentrations (0.4 per cent) of  $\text{CO}_2$  and no  $\text{CO}_2$ , except for small amounts given off in respiration, the experiment being conducted during the latter part of May and early in June. The average temperature was  $25^\circ \text{C}$ . Seedlings of each type were harvested when the culture of the particular type, which had the most limiting conditions for growth, had reached its maximum size. Cultures of each kind, grown with and without  $\text{CO}_2$ , were harvested on the same day.

(a) ON THEIR OWN NITROGEN RESERVES.—Seedlings from low-protein corn, when grown in an atmosphere lacking  $\text{CO}_2$  except for small traces given off by the seedlings themselves in respiration, produced larger plants with greener leaves than did those which had 0.4 per cent  $\text{CO}_2$ . Seedlings from high-protein corn, however, grew more and had greener leaves in an atmosphere containing 0.4 per cent  $\text{CO}_2$ . Seedlings from other types of high-protein seeds, such as soy bean, cowpea, sunflower, and cantaloupe, all responded similarly to the high-protein corn, and were also greener when given  $\text{CO}_2$  than when grown without it. Some kinds of seedlings had their total growth increased nearly 150 per cent by using  $\text{CO}_2$ . Even more striking than the effect of  $\text{CO}_2$  on total growth, is its influence on the shoot and root ratios. Growth of roots was increased much more than growth of shoots in those seedlings whose total growth was increased by  $\text{CO}_2$ . Growth of roots of soy bean seedlings was increased

202 per cent more than that of the tops. From these results it seems that  $\text{CO}_2$  must have had a modifying influence in the utilization of foods stored in the seed.

(b) WITH NITRATES.—Seedlings from low-protein seeds, such as low-protein corn, when grown on their own carbon supply, had their growth increased by the addition of nitrates. Seedlings from high-protein seeds, grown on their own carbon supply, varied in their behavior with respect to the addition of nitrates. Sunflower seedlings grew the same amount with nitrates as without, and muskmelon, cowpea, and soy bean seedlings grew somewhat more with nitrates. When grown in high concentrations (0.4 per cent) of  $\text{CO}_2$ , seedlings of both high and low-protein seeds showed increased growth with nitrates, but those of high-protein seeds had the greater increase. The response in total growth of seedlings of high-protein seeds to  $\text{CO}_2$  in most cases was much greater than their response to nitrates. The response in total growth of seedlings of low-protein seeds to  $\text{CO}_2$  was much less than their response to nitrates.

### 3. Seedlings grown in light in normal atmosphere

This experiment was conducted in October, the plants being grown in the greenhouse in daylight at an average temperature of  $24^\circ\text{C}$ . There were many cloudy days during the progress of the experiment. Each kind of seedling was allowed to grow until the seedlings of the lot grown without nitrates had reached their maximum size. The seedlings grown with and without nitrates for each type used were harvested at the same time.

(a) ON THEIR OWN NITROGEN RESERVES.—In most cases there was a greater total growth in light than there had been in darkness in the experiments previously described. Low-protein wheat, however, grew as much in darkness as in light; low-protein corn grew 45 per cent less in light than in darkness; and rice grew 43 per cent less in light than in darkness. Seedlings from high-protein seeds, on the contrary, grew much more in light than in darkness, and some of them produced blossom buds. Greater than the differences in total growth, however, were the differences in shoot to root ratios, relatively more roots being produced in light than in darkness. The greatest degree of shifting of ratios occurred in the seedlings of high-

protein seeds. With the exception of seedlings of the Gramineae, there were notable increases in leaf development in the light, as indicated by size, weight, and number of leaves.

(b) WITH NITRATES.—The seedlings may be classified into three groups with respect to their response to nitrates: seedlings of legumes, seedlings from low-protein and high-protein starchy seeds, and seedlings from high-protein, oily seeds. Seedlings of the leguminous type were the least responsive to nitrates. In general, they did not produce more than 30 per cent more total growth with nitrates than without, under the conditions of light and temperature used in this experiment. In previous experiments, conducted during the longer and sunnier days of May and early June, it had been shown that leguminous seedlings lacking nodules did have the ability to utilize nitrates. The tests with soy bean and cowpea seedlings, however, had indicated that increase of growth with nitrates did not occur to any extent except when photosynthesis of carbohydrates could go on at the same time. For this reason it is supposed that the failure to respond to nitrates during the shorter and somewhat cloudy days of October may be a seasonal condition, and may be connected with the lower light intensity and its resulting effects on photosynthesis of carbohydrates and proteins. Whatever may be the cause, whether difference in light intensity and duration, or temperature, or a combination of these factors, the fact remains that the legumes behaved in this experiment as a group that is characterized by a low grade ability to utilize nitrates.

Seedlings of the low-protein starchy seeds, all members of the grass family, in general produced the greatest response to nitrates in a given time. Low-protein corn gained 194 per cent more than high-protein corn as a result of using nitrates. Low-protein wheat gained 85 per cent more with nitrates than did high-protein wheat. Rice was injured by nitrates. The seedlings grew much larger without nitrates than with them. Seedlings of the high-protein, high-oil type responded more slowly to the influence of nitrates. During the first 10 days of growth, the seedlings with and without nitrates were not greatly different in size. Tomato and sunflower seedlings were exceptions; they showed well marked differences in the earlier stages of growth. It is supposed that the behavior of sunflower

seedlings is explainable because of their rapid response to  $\text{CO}_2$  as discovered in a preceding experiment. It was then noted that sunflower seedlings responded remarkably to  $\text{CO}_2$  even when nitrates were lacking, but that there was no response to nitrates when  $\text{CO}_2$  was lacking. Tomato seedlings have not yet been tested in this way. At the time the plants were harvested, the seedlings of the high-protein, high-oil type presented notable differences when grown with and without nitrates. This was to some extent because they were allowed to grow for a longer time than seedlings of the low-protein, starchy type. In a future experiment it is planned to obtain the weights of the entire lot of seedlings at 5, 10, 15, and 20 day intervals. In this way it is hoped to present the quantitative and qualitative differences in the growth responses at different stages in the development of the seedlings of the various types studied.

With the exception of cotton seedlings, the shoot to root ratios of all types were increased by the addition of nitrates. In general, those seedlings whose total growth was most markedly influenced by nitrates were the ones whose shoot to root ratios showed the greatest increases. Seedlings of the Leguminosae were the least responsive in this respect, as they also were in their response in total growth.

The growth responses of the seedlings to varying proportions of carbohydrates to nitrogen agree with results obtained with tomato cuttings, described in former papers.<sup>2</sup>

### Summary

1. Different kinds of seedlings produce very different growth responses when exposed to the same external influences. The responses are often related to the chemical composition of the seed.

2. If the nitrogen content of the seed is low in proportion to the carbohydrates, the response as measured by an increase in total growth in terms of green weight is greater when nitrates but no  $\text{CO}_2$  (except traces given off in respiration) are utilized than when  $\text{CO}_2$  but no nitrates are utilized.

<sup>2</sup> REID, MARY E., Relation of the kind of food reserves to regeneration in tomato plants. *BOT. GAZ.* 77:103-110. 1924.

———, Quantitative relations of carbohydrates to nitrogen in determining growth responses in tomato cuttings. *BOT. GAZ.* 77:404-418. 1924.

———, Growth of tomato cuttings in relation to stored carbohydrate and nitrogenous compounds. *Amer. Jour. Bot.* 13:1926 (detailed report).



3. If the nitrogen content of the seed is high in proportion to the carbohydrates, the response as measured by an increase in total growth is greater when  $\text{CO}_2$  but no nitrates are utilized than when nitrates but no  $\text{CO}_2$  (except for traces given off in respiration) are utilized.

4. The greatest total amount of growth, however, is produced by seedlings from both high and low-nitrogen seeds by allowing the seedlings to utilize both nitrates and  $\text{CO}_2$ .

5. The utilization of both nitrates and  $\text{CO}_2$  tends to result in a shifting of the relative amounts of shoots and roots, that is, there are qualitative as well as quantitative responses to these external influences. The proportion of shoots to roots of low-protein seeds tends to be increased because of the relatively larger increase in the amount of shoots, if nitrates but no  $\text{CO}_2$  is utilized; whereas the proportions of shoots to roots of seedlings of high-protein seeds are not increased and in some cases are even decreased by this treatment.

6. The shoot to root ratios of seedlings of low-protein seeds are not noticeably affected if  $\text{CO}_2$  but no nitrates are utilized, but the shoot to root ratios of high-protein seeds tend to be greatly reduced if  $\text{CO}_2$  but no nitrates are utilized. This shifting of ratios is a result of a greater increase in the amount of roots than of shoots.

7. The proportions of shoots to roots of seedlings of low-protein seeds tend to be slightly increased if both  $\text{CO}_2$  and  $\text{NO}_3$  are utilized, but the proportions of shoots to roots of seedlings of high-protein seeds are very greatly decreased by such treatment, because the weight and number of roots increase much more than the shoots.

8. The modification of type of growth is correlated with extent of modification in quantity of growth. Those treatments which result in the greatest increase in quantity of growth are the ones which produce the greatest change in type of growth.

(The foregoing statements apply to growth of seedlings in the light.)

9. A high-protein, high-oil food supply appears to be the most efficient in producing growth both in darkness and in light. This is not surprising, since both types of foods are constituted of a relatively large proportion of the condensed compounds of their respective types. For example, high-protein seeds contain relatively much

more of basic nitrogen and consequently have a great amount of nitrogen in proportion to their weight; oils contain a great amount of carbon in proportion to their weight.

10. The shoot to root ratios of seedlings grown in darkness appear to vary with the proportion of nitrogen to carbohydrates in the food reserves of the seed, the higher the nitrogen in proportion to carbohydrate content the higher the shoot to root ratios of the seedlings. There is some evidence that addition of nitrates to the culture medium of the seedlings grown in darkness can modify the type of growth (increase shoot to root ratios), but in only a few cases do nitrates increase the amount of growth.

11. Since wide variations in growth responses are associated with variations in the types and quantities of storage materials in the seed, it seems inadvisable to draw conclusions as to the effect of mineral nutrients or carbon dioxide treatment upon the subsequent growth of the plants when the observations are based on growth responses during the first three weeks of growth only. Before studying the effect of an element or compound on growth (in general), it would seem desirable to conduct experiments in which the storage supply of the element or compound in question has been at least partially exhausted, by allowing the seedlings to grow to maximum size without that substance. This method has been employed by animal workers for some time.

12. Although contradictory results have not been found in the different series of experiments, the results are not considered conclusive but will be repeated and elaborated.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH  
YONKERS, N.Y.

## EXPLANATION OF PLATES XVI-XVIII

### PLATE XVI

FIGS. 1, 2.—Illinois high-protein corn seedlings, grown without nitrates: former without CO<sub>2</sub>, latter with CO<sub>2</sub>.

FIGS. 3, 4.—Illinois high-protein corn seedlings grown with nitrates: former without CO<sub>2</sub>, latter with CO<sub>2</sub>.

FIGS. 5, 6.—Illinois low-protein corn seedlings grown without nitrates: former without CO<sub>2</sub>, latter with CO<sub>2</sub>.

FIGS. 7, 8.—Illinois low-protein corn seedlings grown with nitrates: former without CO<sub>2</sub>, latter with CO<sub>2</sub>.

PLATE XVII

FIGS. 9, 10.—Rocky Ford melon seedlings grown without nitrates: former without CO<sub>2</sub>, latter with CO<sub>2</sub>.

FIGS. 11, 12.—Rocky Ford melon seedlings grown with nitrates: former without CO<sub>2</sub>, latter with CO<sub>2</sub>.

FIGS. 13, 14.—New Era cowpea seedlings grown without nitrates: former without CO<sub>2</sub>, latter with CO<sub>2</sub>.

FIGS. 15, 16.—New Era cowpea seedlings grown with nitrates: former without CO<sub>2</sub>, latter with CO<sub>2</sub>.

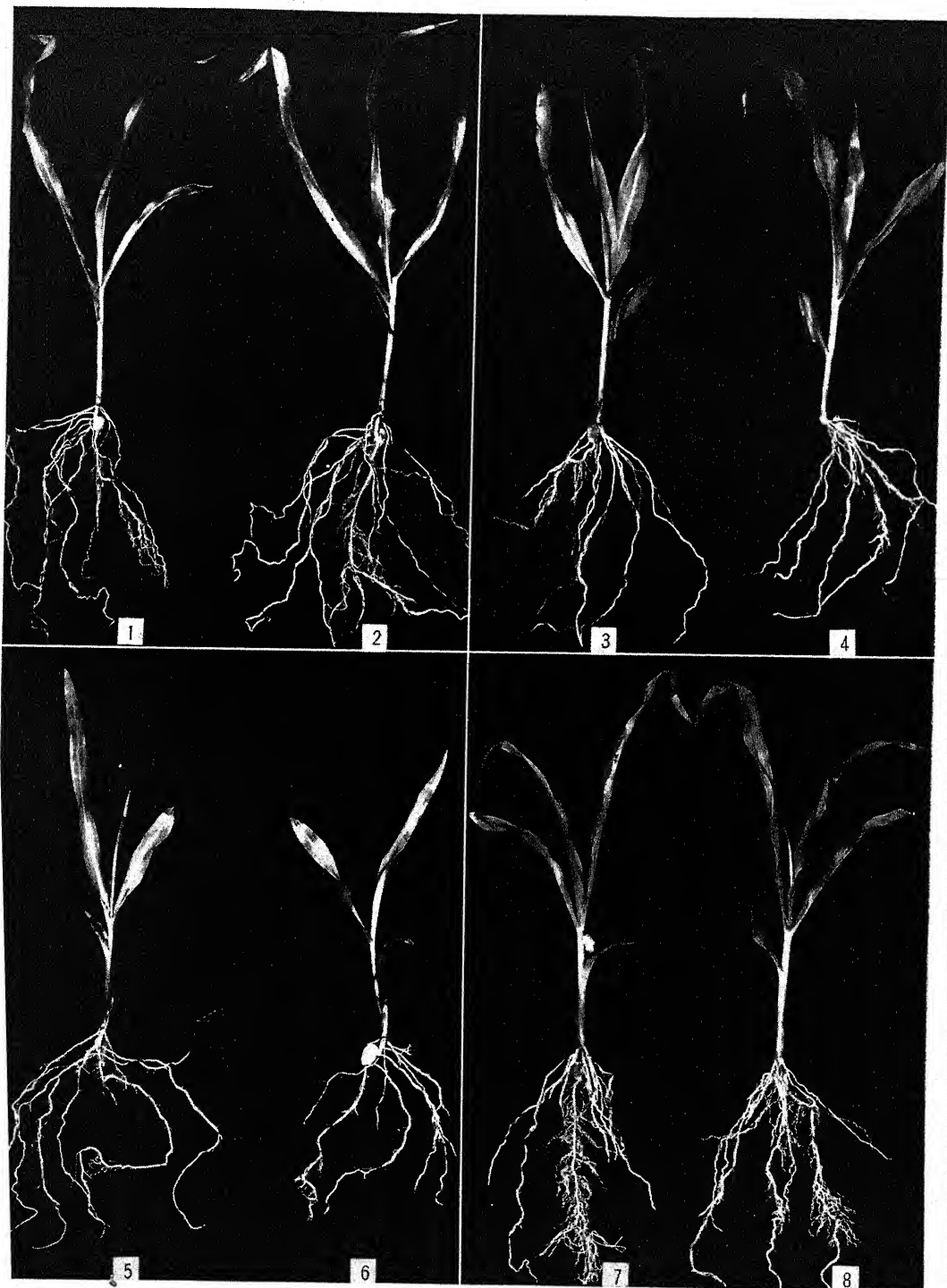
PLATE XVIII

FIGS. 17, 18.—Low-protein (Little Club) wheat seedlings grown in normal atmosphere: former without nitrates, latter with nitrates.

FIGS. 19, 20.—High-protein (Marquis) wheat seedlings grown in normal atmosphere: former without nitrates, latter with nitrates.

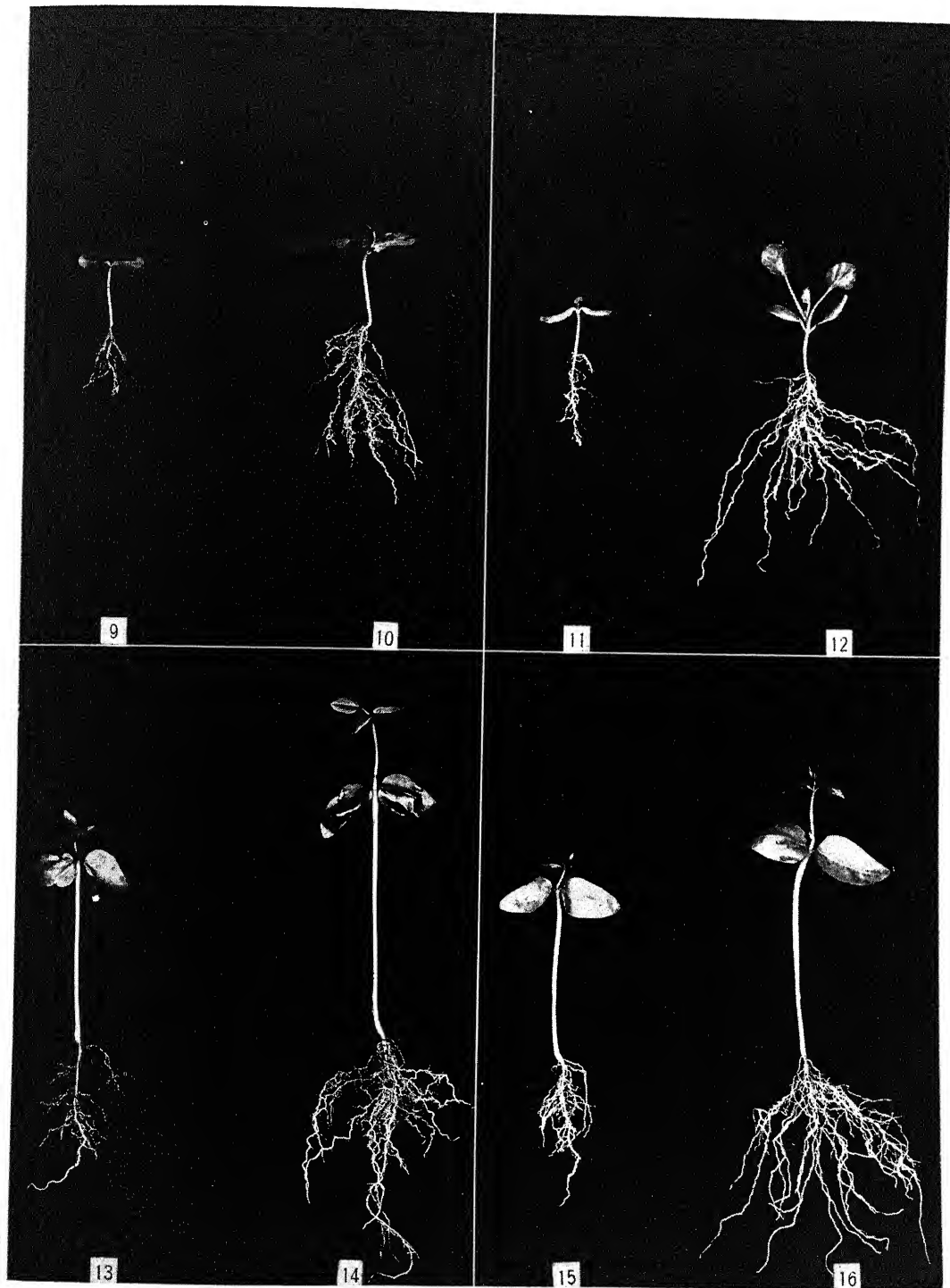
FIGS. 21, 22.—New Era cowpea seedlings grown in normal atmosphere: former without nitrates, latter with nitrates.

FIGS. 23, 24.—Hubbard squash seedlings grown in normal atmosphere: former without nitrates, latter with nitrates.

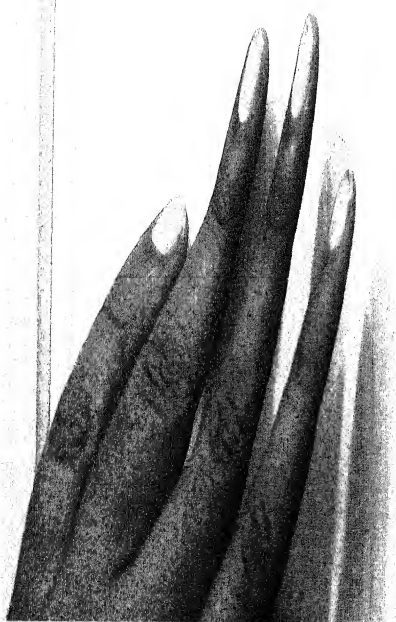


REID on SEEDLING GROWTH





REID on SEEDLING GROWTH





REID on SEEDLING GROWTH





## MICROCHEMICAL AND MORPHOLOGICAL STUDIES OF EFFECT OF LIGHT ON PLANTS

NORMA E. PFEIFFER

(WITH PLATE XV AND FOUR FIGURES)

### Microchemical studies

In recent years there has developed a tendency to correlate with macrochemical analyses as thorough microchemical studies of the fresh tissues as the investigator can make. By the combination of the two methods, the quantitative results obtained by the one can in some degree be checked, and helpful information as to the distribution and localization of the substances involved can be contributed by the other.

There has been slight evidence of this tendency in investigations dealing with the effect of light duration on plants. The earliest work in which artificial illumination was used (MANGON 19, PRILLEUX 23) served to establish production and activity of chlorophyll as in sunlight. There followed a number of investigations in which artificial light of one sort or another, in varying intensities, was used to supplement daylight. In the major portion of this work, possibly because of commercial importance, much attention was given to the effect on the form, size, weight, time of flowering, and seed production of the plant (ADAMS 1, 2, 3, BAILEY 5, CORBETT 7, DEHÉRAIN 9, HARVEY 15, OAKLEY and WESTOVER 22, SIEMENS 24, TIEDJENS 25, WANN 26, WANSER 27). In relatively few investigations (GARNER and ALLARD 12, 13, LUBIMENKO and SZEGLOFF 18, NIGHTINGALE 21) the chemical background for these external changes was touched upon. TIEDJENS (25) briefly tested for carbohydrates, and NIGHTINGALE (21) especially supported his macroanalyses with microchemical data.

It seemed important for more complete knowledge of the situation to apply both macrochemical (ARTHUR 4) and microchemical methods to the situations occurring in plants grown in different daily periods.

## CONDITIONS OF PRESENT EXPERIMENT

The light conditions varied in intensity and amount in the different sets. In one room plants were grown in artificial light supplied by twenty-five 1500-watt lamps, with an intensity varying from 780 foot candles at the beginning of the run to 352 foot candles at the end, as measured by the Macbeth Illuminometer.<sup>1</sup> As determined by the pyrhelimeter, the measurement at the beginning was 2.406 gm. calories per sq. cm. per minute, and 1.938 gm. calories at the end. The daily periods of illumination were for 5, 7, 12, 17, 19, and 24 hours for the different sets of plants in this room. There was a fluctuating supply of carbon dioxide, averaging 0.3 per cent. The temperature was constant at 25.5° C., and the humidity at 80 per cent of saturation.

Plants in two houses equipped with a gantry crane<sup>2</sup> carrying forty-eight 1000-watt lamps, had 6 hours of additional illumination beyond natural daylight. In one case this artificial light was supplied from 6:00 P.M. to midnight, in the other from midnight to 6:00 A.M. The former had an extra supply of carbon dioxide, the latter the usual atmosphere. In both cases the artificial light was 383.69 foot candles (Macbeth Illuminometer) and 0.465 gm. calories per sq. cm. per minute (pyrhelimeter) at the beginning of the experiment, and gradually diminished to 141.5 foot candles (Macbeth Illuminometer) and 0.323 gm. calories (pyrhelimeter) at the end. A house with ordinary atmosphere and the natural light conditions of the season (March, April, and beginning of May) served for the control plants. The temperature and humidity were as in the continuous light room.

## METHODS

From previous experience it was deemed that an intensive study of the tomato (*Lycopersicum esculentum*), which shows a decided reaction to longer periods of light, would be of importance. Studies were made of individual plants of the variety Bonny Best in each of the series in different daily periods of illumination, at various in-

<sup>1</sup> Data supplied by J. M. ARTHUR.

<sup>2</sup> Greenhouses equipped with gantry crane are described and illustrated in Contr. Boyce Thompson Inst. Plant Research 1:17. figs. 5, 6. 1925.

TABLE I  
TOMATO; MICROCHEMICAL ANALYSES

HOURS LIGHT	AGE (DAYS)	SUGARS	STARCH	BIURET	NITRATES	PHOSPHATES	MAGNESIUM	GENERAL RATIO
5	7	Very little	Very little	Good	Very high	.....	.....	Low carbohydrates: low protein
	15	Very little	Very little	Little	Much	.....	.....	
	25	Very little	Very little	Little	Excessive	Negative	Negative	
7	43	Increase	.....	.....	Excessive	.....	.....	Low carbohydrates: low protein
	7	Very little	Very little	Little	Very high	.....	.....	
	16	Little	Little	Little	Much	.....	.....	
12	28	Little	Little	Little	Excessive	.....	.....	Slightly increased carbohydrates: slightly increased protein
	44	Little	Little	Little	Excessive	Very little	Negative	
	6	Little	Moderate	Little+	Very high	.....	.....	
	16	Little	Decrease	Little+	Much	.....	.....	
	25	Little	Decrease	Negative	Excessive	.....	.....	
17	44	Increase	Little	Little	Moderate	Negative	Negative or trace	Higher carbohydrates: protein as in 12 hours
	8	Little	Little	Moderate	Very much	.....	.....	
	16	Little	Little	Moderate	Much	.....	.....	
	24	Increase	Increase	Little	Excessive	.....	.....	
	38	Greater increase	Increase	Little	Moderate or negative	Very little	Trace to fair	
19	8	Little	Little	Moderate	Very much	.....	.....	Low carbohydrates: low protein
	16	Slight increase	Little	Little	Much	.....	.....	
	24	Same	Little	Little	Excessive	Negative or little	Little to much	
24	37	Increase	Slight increase	Decrease	Excessive	.....	.....	Slightly increased carbohydrates: higher protein
	9	Little	Little	Moderate	Excessive	.....	.....	
	17	Little	Little	Little+	Very much	.....	.....	
	25	Little	Little	Little+	Excessive	.....	.....	
	48	Great increase	Moderate	Little to good	Negative	Negative or little	Fair	
Control	7	Negative	Little	Good	Excessive	.....	.....	Moderate carbohydrates: lower protein
	18	Very little	Fair+	Fair	Excessive	.....	.....	
	27	Increase	Fair+	Little	Excessive	.....	.....	
Gantry crane house, +CO <sub>2</sub>	45	Same	Moderate	Decrease	Negative	Negative or trace	Moderate	Moderate carbohydrates: low protein
	7	Very little	Very little	Little+	Excessive	.....	.....	
	16	Fair	Fair	Little+	Excessive	.....	.....	
	35	Moderate	Moderate	Little+	Negative	Fair	Moderate	
	8	Negative	Little	Fair	Excessive	.....	.....	
Gantry crane house, usual atmosphere	10	Fair	Little+	Little	Excessive	.....	.....	Moderate carbohydrates: less protein
	18	Moderate	Fair	Very little to little	Trace	Negative	Much	
	35	Moderate	Fair	.....	.....	.....	.....	

tervals, until the plants were about 6 weeks old. Analyses were made at as near the end of the daily exposures to light as possible. Tests were made for the carbohydrates, protein, nitrates, magnesium, calcium, and phosphorus, on free-hand sections in each of 5 regions of the plant, namely, root, lower stem, middle stem, upper stem, and leaf, with the object of obtaining an idea of the plant chemistry in different regions. Aside from this intensive study of a single form, buckwheat (*Fagopyrum esculentum*) of Henderson's Japanese variety was considered, with a longer interval between analyses, and a few other forms were examined for supplementary data at but one time. Unfortunately, in this phase of the work there is some error due to individual differences, since there were not enough plants of a single kind to avoid this difficulty.

Standard tests were used, Flückiger's reaction for fructose, glucose, and dextrin; potassium iodide solution of iodine for starch; a similar solution and biuret test for proteins; diphenylamine in 75 per cent sulphuric acid for nitrates; formation of ammonium magnesium phosphate crystals for magnesium and phosphorus, by use of appropriate reagents in each case; and 5 per cent sulphuric acid for calcium.

## RESULTS

In the case of tomato, the results of tests, made at four intervals until the plants were about 6 weeks old, can be shown best in tabular form (table I), giving the general conclusion as to the content of the plant from a study of all the regions. This table does not take into account the amount of tissue development, which is more properly considered under the anatomical discussion. Similar tables bring out the important points in regard to buckwheat (table II), as determined at two stages, when the plants were about two and a half weeks old and 7 weeks old. In four o'clock (*Mirabilis Jalapa*) (table III) the aerial part only was tested when the plants were about 5 weeks old. At the conclusion of the experiment, when about 10 weeks old, the roots were examined. The table dealing with the earlier tests shows the part of the body commonly used for gross analyses; the latter, dealing with the organ commonly overlooked, gives interesting data in regard to reserves in different light durations (see also morphology). Observations on other plants, although too scat-

TABLE II  
BUCKWHEAT; MICROCHEMICAL ANALYSES

HOURS LIGHT	AP- PROXI- MATE AGE (DAYS)	SUGARS	STARCH	BURET	NITRATES	PHOSPHATES	MAGNESIUM	GENERAL RATIO
5	17 48	Little Decrease	Little Increase	Fair Little	Much Excessive	Negative	Trace	Low carbohydrates: low protein
7	17 48	Little Decrease	Moderate Increase	Little + Decrease	Much Excessive	Excessive	Excessive	Low carbohydrates: low protein
12	17 48	Little Decrease	Fair Increase	Moderate Little	Very much Excessive	Much	Excessive	Low carbohydrates: low protein
17	17 48	Little Increase	Moderate Increase	Fair Little	Much Negative	Trace	Little	High carbohydrates: low protein
19	17 48	Little + Increase	Moderate + Increase	Fair Little --	Much Negative	Moderate	Much	High carbohydrates: low protein
24	17 48	Little + Slight increase	Little + Good increase	Fair Fair	Much Trace	Fair	Very much	High carbohydrates: more protein (than 17 or 19 hours)
Control	17 48	Little + Decrease	Little + Decrease	Moderate Little	Much Excessive	Much	Moderate +	Low carbohydrates: low protein
Gantry crane house, + CO <sub>2</sub>	17 48	Little + Increase	Fair Increase	Fair Decrease	Much Trace	Much	Little	High carbohydrates: low protein
Gantry crane house, usual atmosphere	17 48	Little -- Very slight increase	Fair Slight decrease	Fair Slight decrease	Much Fair	Fair	Fair +	High carbohydrates: fair protein

TABLE III  
FOUR O'CLOCK; MICROCHEMICAL ANALYSES

HOURS LIGHT	AP- PROXI- MATE AGE (WEEKS)	SUGARS	STARCH	BIURET	NITRATES	PHOSPHATES	MAGNESIUM	GENERAL RATIO
Aerial parts								
5	5	Very little	Little	Much	Excessive	Little	Little	Low carbohydrates: high protein
7	5	Little	Little	Much	Very much	Very little	Moderate	Low carbohydrates: high protein
12	5	Slight increase	Slight increase	Much	Excessive	Negative	Much	Low carbohydrates: high protein
17	5	As above	As above	Slight increase	Excessive	Much	Very much	Slightly more carbohydrates: high protein
19	5	Fair	Little	Moderate	Excessive	Much	Very much	Slightly more carbohydrates: high protein
24	5	Moderate	Little	Fair	Excessive	Negative	Moderate	Higher carbohydrates: low protein
Control	5	Very little	Little	Moderate+	Excessive	Very little	Moderate	Low carbohydrates: high protein
Gantry crane house, +CO <sub>2</sub>	5	Fair	Fair+	Moderate+	Excessive	Negative	Moderate+	Higher carbohydrates: high protein
Gantry crane house, usual atmosphere	5	Moderate	Moderate	Moderate+	Excessive	Negative	Moderate	Higher carbohydrates: high protein
Roots								
5	10	Negative	Moderate	Fair	Excessive	Negative	Negative	Low carbohydrates: low protein
7	10	Negative	Much	Fair	Excessive	Negative	Negative	Low carbohydrates: low protein
12	10	Very little	Very much	Fair -	Excessive	Trace	Negative	Higher carbohydrates: low protein
17	10	Little	Excessive	Fair	Fair	Moderate	Fair	High carbohydrates: low protein
19	10	Trace	Excessive	Fair	Fair	Much	Little	High carbohydrates: low protein
24	10	Negative	Much	Fair -	Very much	Fair	Much	Less high carbohydrates: low protein
Control	10	Very little	Excessive	Fair +	Fair -	Much	Much	High carbohydrates: low protein
Gantry crane house, +CO <sub>2</sub>	10	Little	Excessive	Fair -	Very much	Fair	Much	High carbohydrates: low protein
Gantry crane house usual atmosphere	10							

tered to be worthy of tabulation, are depended upon in the following discussion.

### DISCUSSION

In coordinating the facts observed, one finds that usually in the short exposures to light there is a low carbohydrate and low protein content, which is coupled, as may be seen in the anatomical work, with relatively less tissue production than in longer exposures. The most marked exception to this ratio is seen in the four o'clock, where the aerial parts early show a relatively higher protein content. In the longer exposures, under similar atmospheric conditions, there is evident a tendency toward greater production of carbohydrates without a proportionately increased ability to utilize the manufactured product in protein formation and tissue development. In tomato and four o'clock this increase, evident in the 12-hour exposure, is even clearer in 17 hours of light. The tomato suffers injury, however, in this and higher durations, with the result that photosynthetic ability falls markedly, so that in 19 hours the carbohydrates are again relatively low. A comparable fall does not appear in the buckwheat, which seems better able to utilize longer exposures. In all cases, except possibly four o'clock, there is a greater carbohydrate content in the plants in the gantry crane house with extra carbon dioxide than in those with the ordinary atmosphere.

In comparisons of the nitrates present in the different sets of conditions in different plants, variation is evident at once. The tomato in longer exposures, as in shorter, shows great amounts of nitrate present. Only in the control and in the two houses receiving extra light beyond daylight are there negative results or only traces. In four o'clock, on the other hand, there are ample nitrates in the aerial part in all series, although the subterranean parts show much less at the end of the run in 17, 19, and especially in 24 hours' exposures. Practically the same thing is true in buckwheat as a whole as in four o'clock roots. In other forms examined, it was found that usually the nitrates ran lowest in the gantry house with extra carbon dioxide. There is a possibility that the plants in this series, making the great growth that they do, draw too heavily on the soil, and may not have adequate available nitrates at the end of the run. This point will be guarded more carefully in further work. It may be



pointed out, however, that such low nitrate content in these plants was found at various stages in several plants, and so was not usually due to low soil supply.

Data in regard to the occurrence of phosphates and magnesium are as yet inadequate for drawing general conclusions. Both appear relatively lower as a rule in the shorter exposures, due probably to proportionately greater utilization in production of proteins and tissue formation.

### Anatomical studies

#### LITERATURE

The most comprehensive study of the anatomical differences brought about by artificial light was made by BONNIER (6). Using arc lights as continuous light or for periods of 6 hours in the morning and 6 hours in the afternoon, with fairly constant temperature and humidity, he grew a wide variety of forms and made studies of stem and leaf characters. He drew the following conclusions in regard to the effect of continuous light: (1) the chlorophyll is greater in amount and more uniformly distributed in all the cells containing it in the normal plant; (2) the leaf blade structure is simplified, that is, the palisade is less distinct or entirely lacking and the epidermis has thinner walls; (3) the stem structure is simplified, with late or poorly developed cork, less distinct endodermis, less lignification and sclerification. He also found that the structures in discontinuous electric light were more like those in discontinuous solar light than those in continuous electric illumination. He thinks of continuous light as producing a result much like etiolation except for greater greenness.

LUBIMENKO and SZEGLOFF (18), dealing with periods of light from 14 hours down to 4, found greater concentration of chlorophyll in the longer exposure, by spectrophotometric methods rather than by chloroplast study.

More recently, MASSART (20) studied the effect of continuous light on leaf structure. With a range of forms including representatives from Hepaticae, *Selaginella*, and flowering plants, he used intervals of 6, 12, 18, and 24 hours' exposure to light. He comes to the conclusion that effects on form and structure are more dependent on

intensity than on duration, and that in the intervals used, continuous and discontinuous light affect assimilatory organs in the same way.

Miss DEATS (8), in her anatomical studies of pepper and tomato grown in intervals of 6.5 hours (short day), 17.5 hours (long day), and the normal day period, found that the amounts of bast and xylem in stems varied directly with the length of day. There was variation in the thickness of their cell walls in the same direction. Similarly the size of the epidermal cells and the amount of cork varied directly with the duration of light. In leaves, the greatest thickness and size and depth of greenness occurred in long day plants, the least in short day.

### RESULTS

STEM STRUCTURE.—An attempt was made to follow the degree of development of the stem whenever sections were made for microchemical studies. Inasmuch as only a single specimen was used each time, there is bound to be individual variation in the results. The plants were not uniform in development, and one must be aware of this fact.

It seemed that the structure near the base of the stem is the most conservative that one could use for demonstration of results, since the region used could be more exactly determined as a definite distance from soil surface. "Middle stem" is less exact, since plants attained different heights in different light intervals, and the "middle" would be at various levels. Similarly the tip of the stem and the roots, which varied from plant to plant, seem to add less to our knowledge of how the light affected the plant than the base of the stem. I therefore made diagrams of this region in tomato (text figs. 1, 2) and buckwheat (text fig. 3), to show the relative amounts of tissue developed in the different sets of conditions. In tomato these correspond roughly to each other in the different series, sometimes with variation of several days between comparable examinations. This variation and individual variation must both be considered in appreciating how well such diagrams portray the actual effect. Text figs. 1 and 2 show the stems at the time of the first, third, and fourth microchemical analyses (compare table I) in all series up to 24 hours' illumination; of the first, second, and third in the gantry crane

houses; and first, second, and fourth in the control set. It should be borne in mind, therefore, that the second diagram of the control shows a younger plant (7 days' difference) than most of the series,

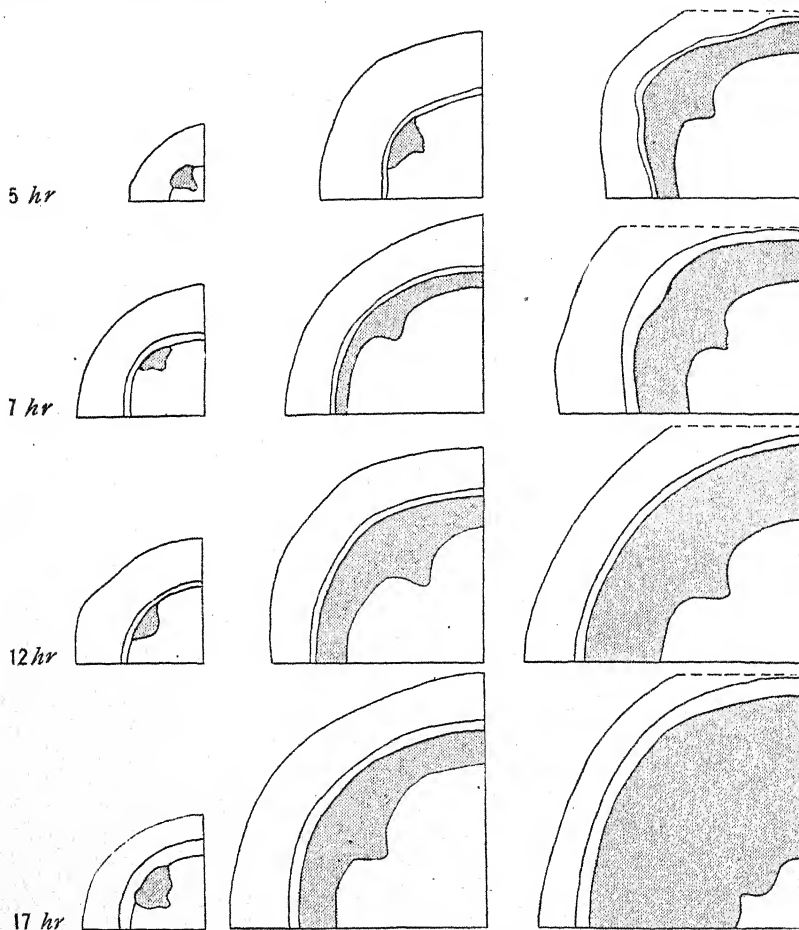


FIG. 1.—Diagrams of bases of stems of tomatoes grown in daily light durations of 5, 7, 12, and 17 hours, showing proportions of tissues at time of first, third, and fourth microchemical analyses in columns 1, 2, and 3 respectively; xylem shaded; compare table I.

and that the two gantry crane house plants were not so old in their last pictured stage as all other series.

In buckwheat there is better correspondence in the stages, since only two analyses were made, with diagrams at these stages.

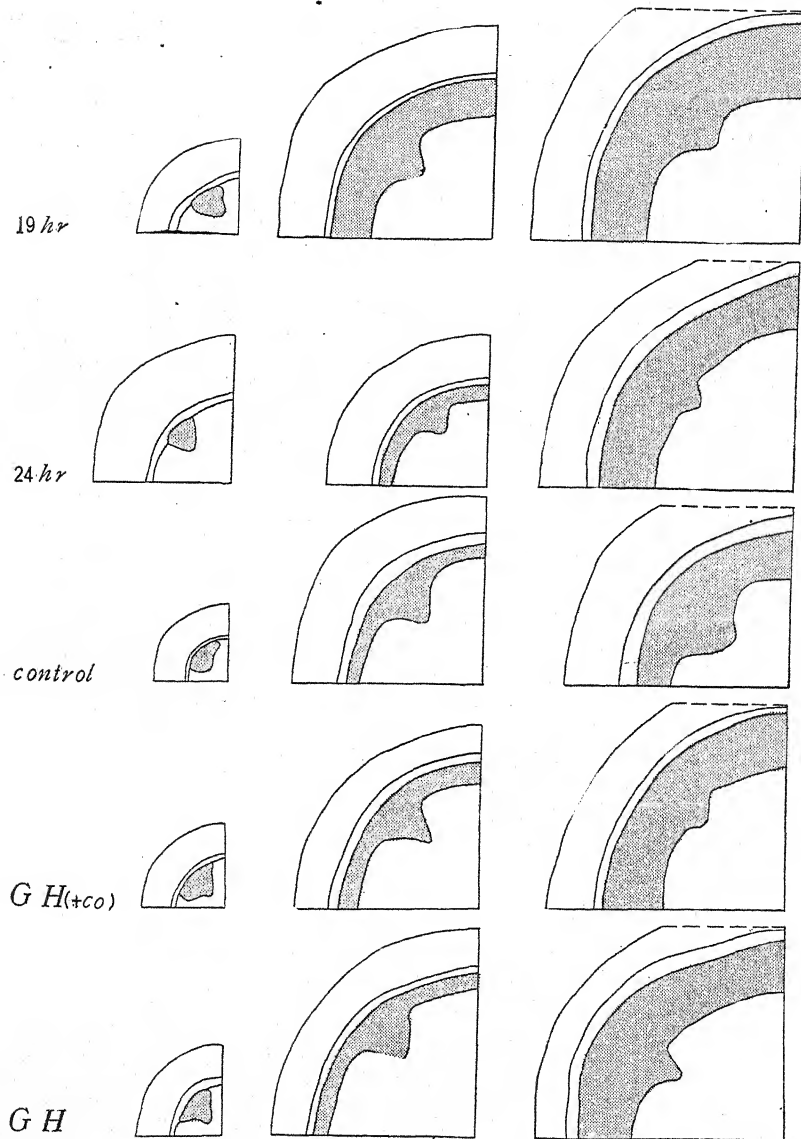


FIG. 2.—Diagrams of bases of stems of tomatoes grown in daily light durations of 19 and 24 hours, in control house (normal light of season), and in gantry crane houses with extra carbon dioxide and usual atmosphere; first, second, and third columns correspond, in 19 and 24 hour sets, to first, third, and fourth microchemical analyses respectively; in control, to first, second, and fourth microchemical analyses; in gantry crane houses, to first, second, and third microchemical analyses; compare table I.

In tomato all series with light duration of 12 hours or more attain as great a diameter or greater than the control, regardless of the

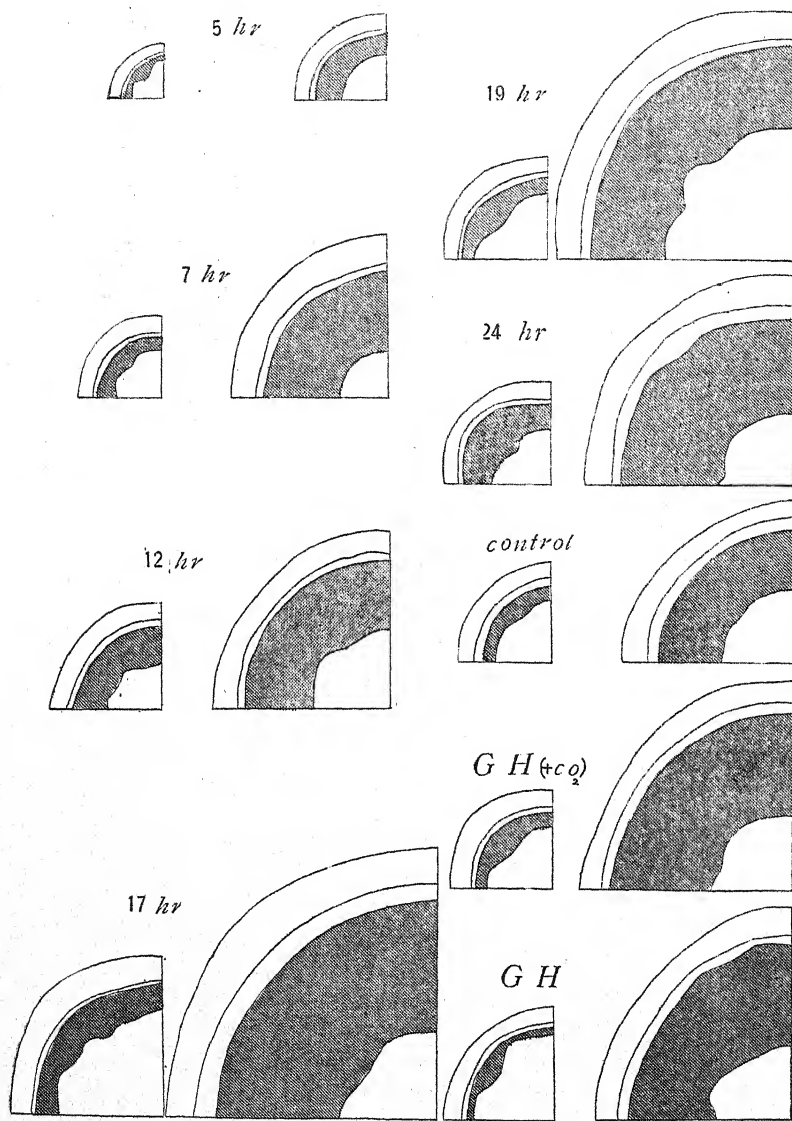


FIG. 3.—Diagrams of bases of stems of buckwheat in different sets of experiment: in each couple of diagrams the smaller shows condition at 17 days, larger at 48 days; compare table II with microchemical data.

success of the plant in other respects; but in several cases, as the 12 and 17-hour plants, there is a very pronounced development of xylem tissue, with relatively less cortex and pith contributing to the diameter. The 12-hour plant was rather diffuse in habit, with somewhat yellow-green leaves, and attained the greatest height of any series. Correlated with the chemical data, this series, having as reserves more carbohydrates and more protein than the lower series, was also utilizing more of these in upward growth than any other set, and gave good evidence in cross-sections of their utilization in highly differentiated rather than simple tissues.

The 17-hour plant, in comparison, although evidently producing much differentiated tissue, was only about three-fourths as tall, had lost all its lower leaves up to the seventh internode, and had a decidedly yellow aspect in the small bladed leaves remaining. It was evidently suffering, as were the plants with longer light durations, from the conditions under which it grew. At the time of cessation of the experiment it showed more carbohydrate reserve, but less protein than the 12-hour form. Considering the condition of the leaves, it is entirely likely that the plant would soon have reached the stage which the continuous light plant attained at about the termination of the run, and succumbed. The 19-hour and continuous light plants were successively shorter than the 17-hour, with progressively poorer physical appearance, as evinced by loss of leaves, yellowing, and, in the case of the 24-hour exposure, by the production of brown areas on leaves, petioles, and stems. Analyzed, these patches (fig. 20) show a sort of cork structure; sometimes similar brown patches are found inside the stem tissues. These give a cellulose reaction when tested, and fail to show tannins.

The gantry crane house plants showed somewhat similar results in tissue development when compared with one another, but the plants supplied with extra carbon dioxide were taller than the others. Compared microchemically, the latter had less reserve protein than the former, and both had only moderate reserve carbohydrates.

As might be expected, the plants with only 5 or 7 hours of light were smaller, very open in habit, with small blades as compared with the total size of the leaves. They were also very watery, and the thin leaves wilted readily. There was little development of the character-

istic complex oil that gives the plant its odor, in these or in the plants with 17, 19, or 24 hours of light.

In buckwheat the maximum diameter was attained in the plants grown in 17 hours of light. These obviously developed the greatest amount of xylem, and showed the greatest height, except for the plant in the gantry house with the usual atmosphere, which made it a close second. The shorter exposures produced correspondingly shorter plants, and correspondingly less of the highly differentiated tissues. Longer exposures show a similar succession from this maximum, although in less degree. The 19-hour and continuous light plants are somewhat shorter than the 17-hour, but almost twice as tall as the 7-hour plant. As compared with one another, there was a slight difference in the 19 and 24-hour plants in favor of the latter as regards both height and amount of xylem, which I believe to be due to variation in individuals rather than to direct effect of light.

The gantry crane house plants showed a slightly better development of xylem and bast when provided with extra carbon dioxide. On comparison with the rest of the series, they show less development than the 17-hour plant, and correspond fairly well with those receiving 19 or 24 hours of light.

In relating these facts with the microchemical data, the 5, 7, and 12-hour plants, like the control, showed low carbohydrate and low protein reserves, while those in longer exposures showed much carbohydrate and low protein. At the time of the last analysis all plants were in flower, but only those in 7 and 12 hours had set fruit. It is clear that the buckwheat attains maximum height and development of tissues and reserves in 17 hours' light exposure, in contrast with tomato, which, although attaining maximum tissue differentiation and reserves, fails to make the growth in height and total diameter. In summation of all points observed, the tomato is more successful in 12 hours' exposure.

LEAF STRUCTURE.—*Capsicum annuum* (variety Ruby King), *Coleus* sp., *Glycine Soja* var. Tokyo, Peking, Mandarin, and Biloxi, *Lactuca sativa* var. Mignonette, *Mirabilis Jalapa*, *Nicotiana Tabacum* var. Imported Havana, *Pelargonium* sp., *Solanum Melongena* var. Black Beauty, *Tropaeolum majus* (Grant Flowering Salmon Queen), *Lycopersicum esculentum* var. Bonny Best, and *Viola* sp. were here

considered. In addition, *Brassica oleracea* var. *capitata* (Early Jersey Wakefield) was used in determining the number of stomata.

Measurements of mature leaves in similar positions on the plants were made by means of an ocular micrometer on free-hand sections (table IV). In pepper, coleus, lettuce, tomato, four o'clock, geranium, and nasturtium, continuous light tended to reduce the thickness of the leaf blade to a greater or less degree, as compared with control plants (pl. XV). The effect of increase in length of light period to 19 hours in the gantry crane houses did not produce such uniform results as these. Pepper, coleus, lettuce, tobacco, and three varieties of soy bean (Tokyo, Peking, and Mandarin) showed a decrease in leaf thickness in both houses, whereas geranium, eggplant, nasturtium, tomato, violet, and Biloxi soy bean were thicker leaved in both houses. Four o'clock leaves were evidently thinner in the gantry crane house with ordinary atmosphere, but only very slightly thicker in that supplied with extra carbon dioxide.

When the comparison is made between plants in the two gantry crane houses, it is found that in two cases, nasturtium and Mandarin soy bean, the leaf thickness is the same under the two sets of conditions. In the majority of cases (nine), however, the reduction in leaf thickness is greater in the house where no carbon dioxide is supplied, while in three (tobacco, tomato, and lettuce) the reverse is true. This would tend to show that in the greater number of forms considered, the longer duration favored reduction in leaf thickness, which the increase in carbon dioxide content served to counter-balance in part. The number of forms and the number of individuals considered, however, do not warrant any sweeping generalizations.

In making measurements of leaves, attention was also given to the development and length of the palisade cells (table IV). As one might anticipate, usually there is a correlation, the palisade cells being shortest where the leaf is thinnest, and longest where it is thickest, so that there is shorter palisade in the thinner leaves in continuous light. To this generalization there are occasional exceptions, as the four o'clock and tomato in the gantry crane house with extra carbon dioxide. Obviously, where there is greatest decrease in thickness, the length of cells in the palisade layer may be so reduced that it is little differentiated in size from the adjacent



spongy cells (as coleus in continuous light); or, where a second palisade layer is usually present, this may be lacking (geranium in continuous light), or less marked (Peking soy bean in gantry houses).

TABLE IV  
THICKNESS OF LEAVES AND LENGTH OF PALISADE CELLS

		CONTROL (MM.)	CONTINUOUS LIGHT (MM.)	GANTRY CRANE HOUSE, +CO <sub>2</sub> (MM.)	GANTRY CRANE HOUSE, USUAL ATMOSPHERE (MM.)
Capsicum annuum..	{Leaf	0.28	0.26	0.22	0.208
	{Length	0.12	0.087	0.086	0.086
Coleus sp.....	{Leaf	0.27	0.14	0.21	0.18
	{Palisade	0.06	0.046	0.07	0.049
Glycine Soja var. Biloxi.....	{Leaf	0.35	.....	0.45	0.346
	{Palisade	{0.096	.....	0.157	0.14
		{0.054	.....	0.06	0.06
var. Mandarin....	{Leaf	0.48	.....	0.40	0.40
	{Palisade	{0.20	.....	0.10	0.115
		{0.10	.....	0.09	0.08
var. Peking.....	{Leaf	0.43	.....	0.25	0.21
	{Palisade	{0.14	.....	0.07	0.05
		{0.11	.....	0.04	0.04
var. Tokyo.....	{Leaf	0.34	.....	0.32	0.26
	{Palisade	{0.085	.....	0.085	0.065
		{0.09	.....	0.06	0.065
Lactuca sativa.....	Leaf	0.236	0.138	0.18	0.208
Lycopersicum esculentum.....	{Leaf	0.27	0.24	0.35	0.48
	{Palisade	0.12	0.08	0.10	0.10
Mirabilis Jalapa....	{Leaf	0.27	0.26	0.277	0.22
	{Palisade	0.095	0.08	0.064	0.05
Nicotiana Tabacum..	{Leaf	0.28	.....	0.22	0.27
	{Palisade	0.09	.....	0.08	0.065} double 0.055} palisade
Pelargonium.....	{Leaf	0.229	0.225	0.33	0.31
	{Palisade	{0.05	0.059	0.08	0.08
		{0.038	No 2nd palisade	0.06	0.04
Solanum Melongena..	{Leaf	0.22	.....	0.26	0.23
	{Palisade	0.08	.....	0.16	0.11
Tropaeolum majus...	{Leaf	0.15	0.13	0.17	0.17
	{Palisade	0.047	0.037	0.05	0.05
Viola sp.....	{Leaf	0.17	.....	0.21	0.18
	{Palisade	0.07	.....	0.09	0.076

In regard to the epidermis, specific measurements were not made. From general observations, no marked trend was noted, nor were marked variations evident in the spongy tissue of leaves under different conditions. In the houses with additional light beyond solar light there was an evident increase in hairiness (soy beans), and a yellow appearance to the leaf rather than the good green of the check

plants. In this latter feature, the plants in the usual atmosphere are intermediate in coloring between the control and those with extra carbon dioxide. Evidently the prolonged light brings about some yellowing, which is aggravated by the increase in carbon dioxide. That there is some difference in size and distribution of chloroplasts is clear even to the casual observer, but the data are inadequate for a clear presentation of these points.

**STOMATA.**—Counts were made to determine the number of stomata on both leaf surfaces in six forms grown in four different situa-

TABLE V

COUNTS OF STOMATA PER SQ. MM. OF LEAF SURFACE; AVERAGE OF THIRTY FIELDS

	SURFACE	CONTROL	CONTINUOUS LIGHT	GANTRY CRANE HOUSE, +CO <sub>2</sub>	GANTRY CRANE HOUSE, USUAL ATMOSPHERE
Brassica oleracea var. capitata....	Upper	116	87	149	69
	Lower	161	155	207	126
Capsicum annum.....	Upper	42	15	60	61
	Lower	113	97	232	137
Lactuca sativa.....	Upper	27	39	62	99
	Lower	41	77	47	120
Mirabilis Jalapa.....	Upper	62	12	83	65
	Lower	150	136	152	266
Pelargonium sp.....	Upper	26	15	21	44
	Lower	148	67	68	121
Solanum Melongena.....	Upper	195	.....	85	130
	Lower	242	.....	204	221

tions. Table V shows the calculations for a square millimeter surface based on the averages from counts of thirty fields. The forms are too few to attach great weight to the results, but the latter may be utilized as a starting point for more thorough studies in later experiments. In continuous light, there are approximately uniform results, usually with reduction in number of stomata on both surfaces (table V). In the gantry crane houses there is sometimes an increase, sometimes a decrease, but usually the results are in the same direction for both houses and for both surfaces. This would seem to show that light is more effective as a factor in determining the number of stomata than is carbon dioxide. The gantry crane houses had similar light conditions, but different in intensity as well as duration, from those in continuous light. There is therefore no good basis of comparison for these two sets.

An attempt was made to correlate the number of stomata with thickness of leaves. In the majority of cases where both were determined, there seemed a tendency to an increase in the number of stomata where the leaves were thinner. The number of cases, however, is too small for generalizations.

ROOTS.—Roots of tomato and buckwheat were examined at the time of study of other organs. Although there was not enough material for intensive work, and pot conditions are apt to cause greater variation in root systems than in aerial, nevertheless there was evident correlation with the development of the base of the stem. Since a plant with a fleshy root might give valuable additional data because of storage material, four o'clock roots were examined at the close of the experiment. The short period plants then showed low carbohydrate and low protein reserves and a small volume (as measured by displacement of water). There was a marked increase in volume in 17 and 19-hour plants, which in turn were smaller than the gantry crane house plant with usual atmosphere, the continuous light, and the other gantry crane house plant, which represented the maximum root development. The increase in diameters of roots was roughly comparable with volume, with 0.35 cm. at top for the 5-hour plant and 1.0 cm. for the 7-hour plant. In longer exposures, however, the simple root of low durations gave way to branched, very fleshy systems.

In contrast to the root systems, the aerial part attained the greatest height in 17 hours' exposure, next successively in (1) 19 hours, (2) gantry crane house with increased carbon dioxide, (3) other gantry crane house, and (4) continuous light and 12 hours, which were approximately equal. Text fig. 4 shows the volumes of roots and heights of stems, indicating that a conclusion in regard to the manufacture of materials, based on the stem height alone, would have been very misleading in this form.

## DISCUSSION

A comparison of these results with those of previous workers brings out both similarities and contrasts. In leaves, continuous light produces thinner leaves with less pronounced palisade cells as seen by BONNIER (6). This fails to substantiate MASSART's (20) conclu-

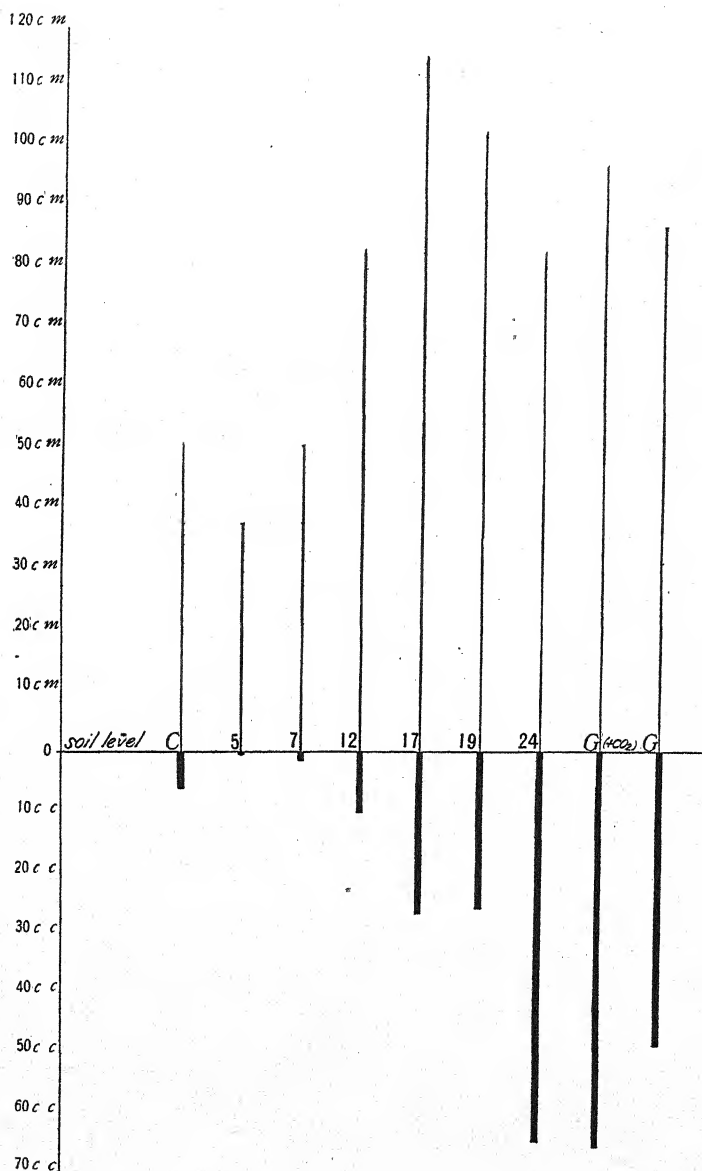


FIG. 4.—Relative development of stem and root regions of four o'clock; lines above "soil level" show height of stem in cm., lines below show volume of roots in cc., successively in control set, in daily light durations of 5, 7, 12, 17, 19, and 24 hours, and in gantry crane house with extra carbon dioxide and with normal atmosphere.

sions in regard to the general similar effect of 24, 18, 12, or indeed 6 hours' illumination on form and structure of leaves. MASSART believes that intensity is more effective in producing structural and form changes, and it may be that differences in intensity are responsible for differences in results obtained by investigators. The earlier records usually include the type of lamp used, range of distances of plants from lamp, but not the measurement of the light falling on the plant.

In long day plants in the gantry crane houses, thinner leaves were produced in the pepper, but thicker in the tomato. The latter is in accord with the work of Miss DEATS (8) on a similar form, the former is not. The presence of extra carbon dioxide might be a factor in one of these houses, but is not active in the other. Further work with more specimens may explain the difference in results.

In regard to stomata, no special work is on record of counts made under similar conditions. A comparison with the results of Miss ECKERSON (11) shows a difference in the control plants, due to conditions of growth in all likelihood. DUFOUR (10) has shown the result of greater intensity of light to be greater number of stomata. In my experiment, the continuous light plant had the lowest light intensity and the lowest number of stomata. Some gantry crane house plants, however, with equal intensity to the control plants during normal daylight plus an additional period of artificial light, also showed reduction in number, tending to remove responsibility from the intensity factor. The matter is complicated by the carbon dioxide supply, which makes an exact comparison with previous work impossible. In correlating with thickness of leaf, I find the usual result in most cases, the larger number of stomata in thinner leaves.

In the stem I failed to find the simplification of tissues reported by BONNIER. There was better agreement with the results of Miss DEATS, who found variation in amount of xylem in short day plants (6.5 hours), normal day plants, and long day (17.5 hours), in direct accord with the length of day. This carries no inference that further increase in time of exposure would further increase the amounts of xylem, under other similar conditions. Indeed my results show for the forms considered that there is a period of exposure for each form, beyond which additional duration is ineffective in producing differ-

entiated tissue, or increased height. The best interval for producing differentiation and height in tomato in artificial light of intensity used was 12 hours, in buckwheat 17 hours.

LUBIMENKO and ŠZEGLOFF (18), in working with a range of shorter exposures (from 14 hours down to 4), found a greater depth of greenness in the longer durations, as did Miss DEATS in her 17.5-hour plants. General observations indicated lower chlorophyll content in the 5 and 7-hour plants than in the intermediate durations, with yellowing occurring to greater or less degree in the 19 and 24-hour exposures. Contrary to this last, BONNIER found greater amounts of chlorophyll in his continuous light plants. The majority of his plants were different from mine, it is not known that he used comparable intensity, and his experiments did not introduce the extra supply of carbon dioxide as a factor. These factors may account for differences.

### Summary

1. In plants with short exposures to light, there are usually low carbohydrate and low protein reserves with less total growth and less production of differentiated tissues than in longer intervals.

2. In plants with longer light duration, there is increase in carbohydrate reserves without proportionately increased use in elaboration of proteins and tissue production.

3. Nitrates are present in great amounts in all tomato plants except the control and gantry crane house specimens. They are low in amount in buckwheat in 17, 19, and 24-hour exposures. They are usually lowest in the gantry crane house with extra carbon dioxide.

4. The maximum development of the plant (considering height and differentiated tissue) occurs in the 12-hour tomato and the 17-hour buckwheat.

5. Injury is evident in the tomato in exposures of 17 hours or more, resulting in marked decrease in photosynthetic ability.

6. Continuous light tends to produce thinner leaves, with palisade layer shorter or lacking.

7. The effect of conditions in the gantry crane houses as regards leaf thickness is variable.

8. Thinner leaves usually show an increase in the number of stomata, except in continuous light in intensity used.

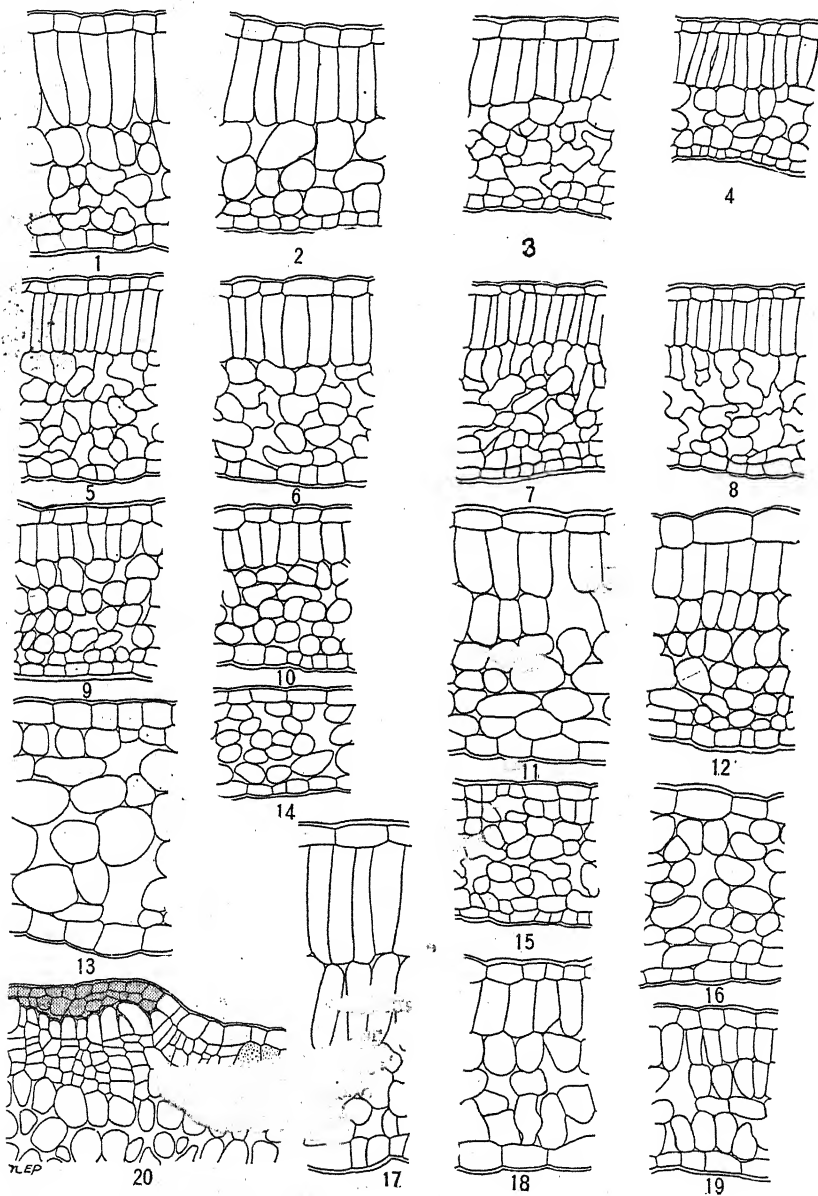
9. Root development in fibrous systems appears roughly comparable with that of the aerial parts.

10. In the storage roots of four o'clock, maximum development occurs in the gantry crane houses and continuous light, while maximum height of stem occurs in the 17-hour plant.

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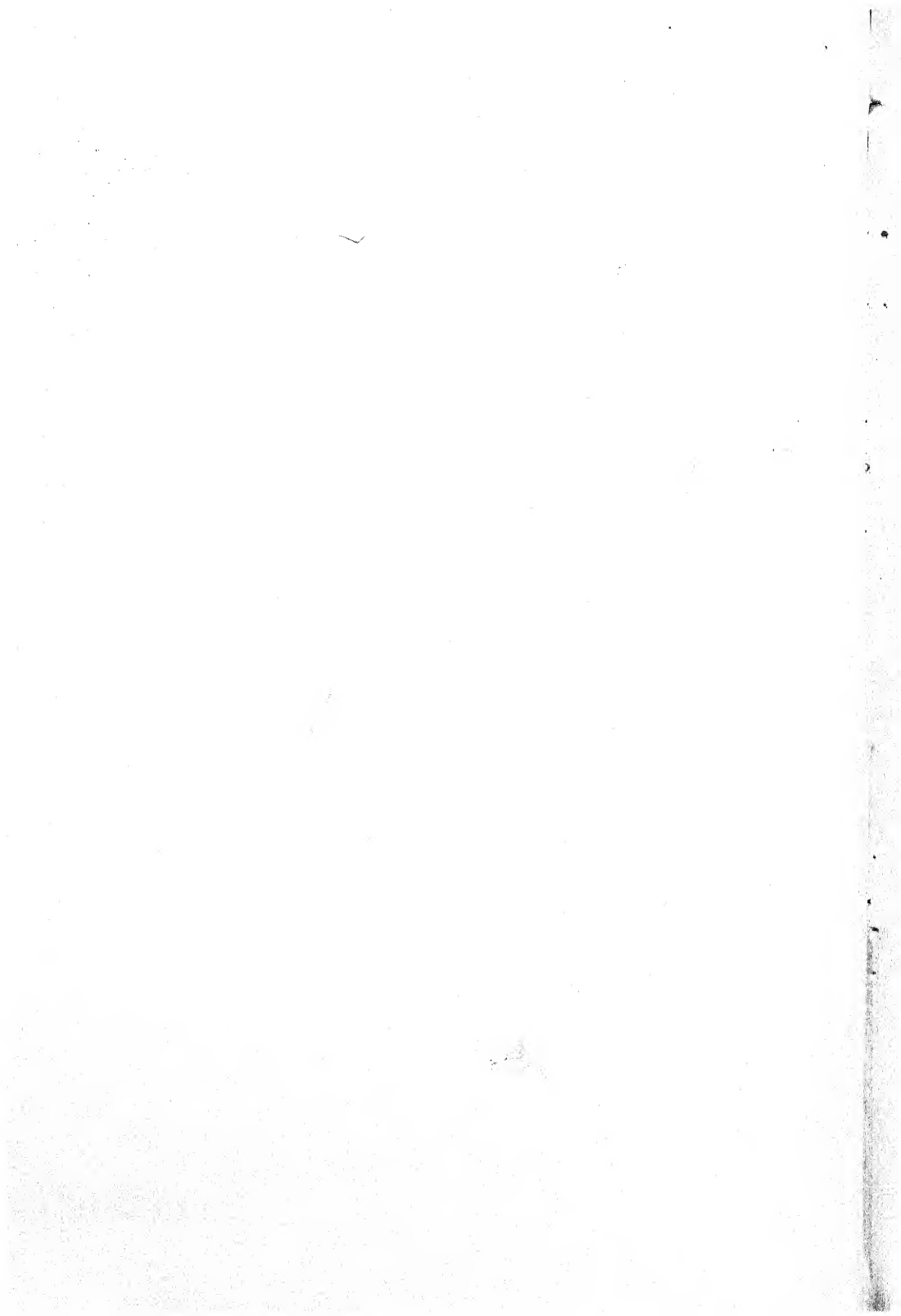
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#### EXPLANATION OF PLATE XV

FIGS. 1-4.—Pepper leaf: successive figures show structure in control, continuous light, and gantry crane house with extra carbon dioxide, and with usual atmosphere.

FIGS. 5-8.—Four o'clock leaf: successive figures as in pepper.

FIGS. 9-12.—Geranium leaf: successive figures as in pepper.

FIGS. 13-16.—Lettuce leaf: successive figures as in pepper.

FIGS. 17-19.—Soy bean var. Peking leaf: successive figures show control, gantry crane house with extra carbon dioxide, and with usual atmosphere.

FIG. 20.—Outer portion of tomato stem, showing injury in continuous light: shaded portion brown in living material; stippled cells are subepidermal ones which have retained green coloring; corklike cells between brown layer and collenchyma tissue.

## ATTEMPT TO CULTIVATE AN ORGANISM FROM TOMATO MOSAIC<sup>1</sup>

HELEN A. PURDY

### Introduction

In discussing the etiology of diseases of man and animal produced by filterable viruses, the consensus of opinion is in favor of the theory that they are caused either by microorganisms not demonstrable by our present methods, or by ultramicroscopic organisms (9, 10, 13, 18). This conclusion is based upon the fact that the active agent in the filtrate apparently multiplies within the living host, since an infection obtained by inoculation with the filtrate can be transferred successively through a series of animals after the dilution of the original inoculum has exceeded the point capable of reproducing the disease. Although the virus of tobacco mosaic exhibits the same property of indefinite multiplication within living plants, not all plant pathologists regard this evidence as sufficient support for the parasitic theory of mosaic diseases.

Many hypotheses have been proposed to explain the origin of tobacco mosaic as a purely physiological response to unfavorable environmental conditions of the soil or climate (3, 5, 7, 14, 15), or due to various agents such as a "contagium vivum fluidum" (1), unorganized ferments or toxins (6, 7), oxidizing enzymes (2, 4, 16, 17), and only recently to potato protoplasm (8). It seems likely that all of these hypotheses would be superseded by the parasitic theory, if the virus could be multiplied *in vitro*.

Many unsuccessful attempts have been made to cultivate a causative organism from plants affected with mosaic disease. Considerable interest, therefore, was aroused when OLITSKY (12) recently reported success in cultivating *in vitro* an active agent that would produce mosaic disease in tobacco and tomato plants. The importance of OLITSKY's conclusions makes a careful repetition of his experiments desirable. The investigations here reported were

<sup>1</sup> The writer is indebted to Dr. L. O. KUNKEL for valuable suggestions and a critical review of the manuscript.

undertaken with the purpose of repeating his recent work. With a few modifications, the methods employed by OLITSKY were carefully duplicated. While this work was in progress, MULVANIA (11) reported that he had repeated OLITSKY's work, but was unable to obtain any evidence that the active agent of mosaic disease multiplies outside the living tobacco and tomato plants.

The stock virus used was extracted from mosaic-affected tomato plants in a greenhouse of the Boyce Thompson Institute. Although OLITSKY obtained his tomato mosaic virus from the same source a year previously, the writer has no proof that the two stock viruses were identical.

### Method

**PREPARATION OF MEDIUM.**—Eighty gm. of tomato shoots, 5-6 weeks old, were minced with scissors and ground to a soft pulp in a sterile mortar; 250 cc. of distilled water added, and the entire mixture centrifuged at high speed for one hour. The supernatant fluid was passed, first through a paper pulp suction filter to remove the bulk of plant tissue, then through a sterile Berkefeld "W" under strictly aseptic conditions, and finally through a second similar Berkefeld filter.<sup>2</sup> The hydrogen-ion concentration of the filtrate was determined by the electrometric method. The reaction of the various lots of medium gave a range of  $P_H$  5.08-6.26.<sup>3</sup> The medium was tubed and incubated at 28°-30° C. for 7 days. At the end of this period of incubation, all tubes showing evidence of contamination or a precipitation of albumins and globulins were discarded. Only the tubes that contained clear media were used.

**TESTING OF MEDIUM.**—At the end of a 7-day period of incubation, a tube of medium was inoculated into 16 healthy tomato plants. After a second week of incubation, the inoculation of 16 additional healthy plants was repeated. If the 32 plants inoculated with a given lot of medium remained healthy, the medium was considered free from virus.<sup>4</sup>

**CULTURE.**—The stem of a badly affected mosaic tomato plant

<sup>2</sup> OLITSKY used a Berkefeld "N" and no paper pulp suction filter.

<sup>3</sup> The media used by OLITSKY gave a reaction of  $P_H$  5.3-6.0.

<sup>4</sup> Considerable difficulty was experienced in obtaining medium free from virus.

was cut with a razor. The cut end was flamed, and a sterile capillary pipette was inserted in the stem, from which approximately 0.01 cc. of juice was withdrawn and inoculated into 5 cc. of medium. The culture was then incubated at 28°-30° C. After 7-14 days, subplants were made by adding 0.5 cc. of the original culture, thoroughly rolled to insure mixing, to 5 cc. of medium. Subsequent subplants were made in a similar manner, and the approximate dilution of the introduced virus was estimated.

**INOCULATION.**—Vigorous tomato plants about 6 weeks old were inoculated by scarifying one leaf on each of three separate branches of the same plant and rubbing in the inoculum with a cork. Cognizant of the fact that a plant, apparently healthy, might already be affected with mosaic disease in its incipient stages, every precaution was taken throughout inoculation to safeguard against a chance transmission of mosaic from one plant to another. The leaves to be inoculated were held by means of a separate paper slip for each plant; the needle used for scarifying the leaves was sterilized by flaming after each inoculation; and an individual sterile cork was employed for rubbing in the inoculum. Moreover, all inoculated plants, including those used for control, were held together for four weeks in the same greenhouse, which was kept carefully fumigated.<sup>5</sup> Under these conditions, all of the plants were exposed equally to the risk of accidental infection.

**CONTROLS.**—In every experiment, in addition to the medium inoculated with virus, two tubes, each containing 5 cc. of medium from the same lot, were included. One was uninoculated; the other inoculated with 0.01 cc. of juice from a healthy tomato plant. These control tubes were treated in every way like the virus cultures. Subplants were made, using the same lot of medium to which the corresponding virus culture had been transplanted. A tube containing 5 cc. of sterile tap water inoculated with 0.01 cc. of juice from a mosaic affected tomato plant was also included in every experiment.<sup>6</sup> Subplants of the water culture corresponding to those of the virus cultures were made in sterile tap water.

<sup>5</sup> Thanks are due FREDERICK E. HEINSOHN for cooperation in keeping the greenhouse free from insect pests.

<sup>6</sup> MULVANIA introduced this control in his repetition of OLITSKY's experiments.

## Results

Of the plants that were inoculated from virus cultures containing original inoculum in an estimated dilution of approximately  $2 \times 10^{-3}$ , 41 of 50, or 82 per cent developed mosaic disease (table I). Subplants from these cultures produced infection in 13 of 70, or 19 per cent of the plants receiving virus in a dilution amounting to  $2 \times 10^{-4}$ . Upon reaching the dilution  $2 \times 10^{-5}$ , the virus cultures infected 9 per cent, or 6 of the 70 plants inoculated. At the next subplant, a  $2 \times 10^{-6}$  dilution was obtained that proved incapable of producing mosaic in any of the 70 plants into which it was introduced.

Of the five separate virus cultures made, three lost their power of infectivity upon reaching a dilution of the original 0.01 cc. of virus approximating  $2 \times 10^{-5}$  or 1-50,000, while the remaining two produced no mosaic at the next subplant, which was approximately a  $2 \times 10^{-6}$  or a 1-500,000 dilution.

The two water cultures lost their power of infectivity at a dilution of the original 0.01 cc. of virus approximating  $2 \times 10^{-5}$  or 1-50,000<sup>7</sup> (table II).

A total of 284 plants used in the control experiments remained healthy. Some were inoculated with plain medium; others with medium inoculated with juice from a healthy tomato plant.

## Discussion

In the tabulated results it will be noted that the power of infectivity of each original virus or water culture and of every subplant in each series was tested and recorded. By this method, any appreciable multiplication of the active agent of mosaic disease may readily be detected. Also, the power of infectivity of the virus cultures can unquestionably be attributed to the 0.01 cc. of virus introduced in the original cultures, since the results of the control experiments prove that all the culture media used were free from any trace of virus. Also, by testing the entire series of subplants of a given culture successively, accidental infection from other sources than contaminated medium can more easily be detected, since a culture producing infection at the fourth subplant, when the third was non-

<sup>7</sup> MULVANIA reported a higher percentage infection from his water cultures than from his virus cultures at a given dilution.

TABLE I

POWER OF INFECTIVITY OF VIRUS CULTURES AT VARIOUS DILUTIONS

CULTURE	PERIOD OF INCUBATION (DAYS)	P <sub>H</sub> OF MEDIUM	APPROXIMATE DILUTION OF ORIGINAL VIRUS	NO. OF PLANTS INOCULATED	NO. OF PLANTS DISEASED*	NO. OF PLANTS HEALTHY*	PER CENT INFECTION	AVERAGE PER CENT INFECTION
A...	14	6.19	$2 \times 10^{-3}$	10	10	0	100	82
B...	14	6.19	$2 \times 10^{-3}$	10†	2	2	50	
C...	7	5.69	$2 \times 10^{-3}$	2	2	0	100	
D...	7	5.69	$2 \times 10^{-3}$	2	1	1	50	
E...	7	5.69	$2 \times 10^{-3}$	2	2	0	100	
C...	14	5.69	$2 \times 10^{-3}$	10	10	0	100	
D...	14	5.69	$2 \times 10^{-3}$	10	6	4	60	
E...	14	5.69	$2 \times 10^{-3}$	10	8	2	80	
A <sup>1</sup> ‡	7	6.19	$2 \times 10^{-4}$	10	5	5	50	19
B <sup>1</sup> ...	7	6.19	$2 \times 10^{-4}$	10	0	10	0	
A <sup>2</sup> ...	14	6.19	$2 \times 10^{-4}$	10	4	6	40	
B <sup>2</sup> ...	14	6.19	$2 \times 10^{-4}$	10	0	10	0	
C <sup>2</sup> ...	14	6.19	$2 \times 10^{-4}$	10	0	10	0	
D <sup>2</sup> ...	14	6.19	$2 \times 10^{-4}$	10	2	8	20	
E <sup>2</sup> ...	14	6.19	$2 \times 10^{-4}$	10	2	8	20	
A <sup>2</sup> §	14	5.69	$2 \times 10^{-5}$	10	0	10	0	9
B <sup>2</sup> ...	14	5.69	$2 \times 10^{-5}$	10	0	10	0	
1A <sup>2</sup> ...	14	5.69	$2 \times 10^{-5}$	10	4	6	40	
1B <sup>2</sup> ...	14	5.69	$2 \times 10^{-5}$	10	0	10	0	
C <sup>2</sup> ...	14	6.19	$2 \times 10^{-5}$	10	2	8	20	
D <sup>2</sup> ...	14	6.19	$2 \times 10^{-5}$	10	0	10	0	
E <sup>2</sup> ...	14	6.19	$2 \times 10^{-5}$	10	0	10	0	
A <sup>3</sup> ...	14	5.69	$2 \times 10^{-6}$	10	0	10	0	0
B <sup>3</sup> ...	14	5.69	$2 \times 10^{-6}$	10	0	10	0	
1A <sup>3</sup> ...	14	5.08	$2 \times 10^{-6}$	10	0	10	0	
1B <sup>3</sup> ...	14	5.08	$2 \times 10^{-6}$	10	0	10	0	
C <sup>3</sup> ...	14	6.10	$2 \times 10^{-6}$	10	0	10	0	
D <sup>3</sup> ...	14	6.10	$2 \times 10^{-6}$	10	0	10	0	
E <sup>3</sup> ...	14	6.10	$2 \times 10^{-6}$	10	0	10	0	
1A <sup>4</sup> ...	14	6.26	$2 \times 10^{-7}$	10	2¶	8	20	4
1B <sup>4</sup> ...	14	6.26	$2 \times 10^{-7}$	10	0	10	0	
C <sup>4</sup> ...	14	5.90	$2 \times 10^{-7}$	10	0	10	0	
D <sup>4</sup> ...	14	5.90	$2 \times 10^{-7}$	10	0	10	0	
E <sup>4</sup> ...	14	5.90	$2 \times 10^{-7}$	10	0	10	0	
1A <sup>5</sup> ...	14	5.90	$2 \times 10^{-8}$	10	0	10	0	0
1B <sup>5</sup> ...	14	5.90	$2 \times 10^{-8}$	10	0	10	0	
C <sup>5</sup> ...	14	5.90	$2 \times 10^{-8}$	10	0	10	0	
D <sup>5</sup> ...	14	5.90	$2 \times 10^{-8}$	10	0	10	0	
E <sup>5</sup> ...	14	5.90	$2 \times 10^{-8}$	10	0	10	0	
Total				366				

\* Final number recorded four weeks after inoculation.

† Six inoculated plants were discarded accidentally a few days after inoculation.

‡ A<sup>2</sup> is the first subplant of A, A<sup>2</sup> is a subplant of A<sup>1</sup>, etc.§ A<sup>2</sup> and 1A<sup>2</sup> are both subplants of A<sup>1</sup>. A<sup>2</sup> was made when A<sup>1</sup> had been incubated one week, 1A<sup>2</sup> after A<sup>1</sup> had been incubated two weeks.

|| Accidental?

¶ These plants were standing beside plants affected with mosaic; doubtless accidental infection since the culture was non-infectious at the preceding dilution.

infectious, would be a questionable result, requiring substantiation before the infection could definitely be attributed to virus present in the culture (table I, footnotes ||, ¶; table II, footnote §).

The fact that the two water cultures did not exhibit the high

TABLE II

POWER OF INFECTIVITY OF WATER CULTURES AT VARIOUS DILUTIONS

CULTURE	PERIOD OF INCUBATION (DAYS)	APPROXIMATE DILUTION OF ORIGINAL VIRUS	NO. OF PLANTS INOCULATED	NO. OF PLANTS DISEASED*	NO. OF PLANTS HEALTHY*	PER CENT INFECTION	AVERAGE PER CENT INFECTION
X.....	14	$2 \times 10^{-3}$	10	2	8	20	9
Y.....	7	$2 \times 10^{-3}$	2	0	2	0	
Y†.....	14	$2 \times 10^{-3}$	10	0	10	0	
X‡.....	7	$2 \times 10^{-4}$	10	0	10	0	6
X‡.....	14	$2 \times 10^{-4}$	10	0	8	0	
Y‡.....	14	$2 \times 10^{-4}$	10	2§	10	20	
X²  .....	14	$2 \times 10^{-5}$	10	0	10	0	0
1X²¶.....	14	$2 \times 10^{-5}$	10	0	10	0	
Y².....	14	$2 \times 10^{-5}$	10	0	10	0	
X³.....	14	$2 \times 10^{-6}$	10	0	10	0	0
1X³.....	14	$2 \times 10^{-6}$	10	0	10	0	
Y³.....	14	$2 \times 10^{-6}$	10	0	10	0	
1X⁴.....	14	$2 \times 10^{-7}$	10	0	10	0	0
Y⁴.....	14	$2 \times 10^{-7}$	10	0	10	0	
1X⁵.....	14	$2 \times 10^{-8}$	10	0	10	0	0
Y⁵.....	14	$2 \times 10^{-8}$	10	0	10	0	
Total			152				

\* Final number recorded four weeks after inoculation.

† This culture is a portion of the preceding one which has been incubated one week longer.

‡ X² is the first subplant of X, X³ is a subplant of X², etc.

§ Accidental?

|| Subplant of X² after incubating X² for one week.

¶ Subplant of X² after incubating X² for two weeks.

power of infectivity that the virus cultures of corresponding dilutions showed, cannot be explained adequately by assuming on the one hand that the tap water had an inhibiting or perhaps deleterious effect upon the active agent of the fresh virus, or by claiming on the other hand a slight multiplication of the virus in the medium used in the virus cultures. The number of water and virus cultures tested is too small, and the method of introducing the original inoculum does not permit of enough accuracy for fair comparisons. More significant



conclusions can be drawn by comparing the percentage infection exhibited by successive subplants of the same series.

### Conclusion

In the experiments here reported, in which the methods of OLITSKY were followed, the writer has been unable to obtain any evidence that the active agent producing mosaic disease in tobacco and tomato plants multiplies outside the living plants.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH  
YONKERS, N.Y.

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## EFFECT OF THIOUREA UPON BUD INHIBITION AND APICAL DOMINANCE OF POTATO<sup>1</sup>

F. E. DENNY

(WITH SEVEN FIGURES)

### Introduction

At each "eye" of the potato (*Solanum tuberosum*) are the rudiments of several buds (three or more, according to ARTSCHWAGER 3). Usually only one of these buds will develop, the growth of the accessory buds being inhibited. If the sprout which first starts is removed, however, then one of the other buds will begin active growth. Whatever may be the fundamental cause of the repression of these supernumerary buds by the dominant bud, it is shown in this paper that treating the cut tuber with a solution of thiourea ( $\text{NH}_2\text{CSNH}_2$ ) causes the growth of two or more (often four or five) buds from a single eye.

The tuber of *Solanum tuberosum* is morphologically a thickened stem, the "eyes" being the much shortened or collapsed lateral branches (APPLEMAN 1). Furthermore, these eyes are located on the tuber in a definitely formed spiral (ARTSCHWAGER 3), comparable to the arrangement on an ordinary leafy twig. In agreement with most stems in young stages, the tubers of most varieties of potato exhibit apical dominance, that is, the ability of the apical bud to prevent the growth of basal buds (APPLEMAN 2). Whatever may be the mechanism by which this dominance of the apical bud is exerted, it is shown in this paper that a treatment with a solution of thiourea prevents the apical bud from completely inhibiting basal buds, and that under certain conditions the direction of dominance may be reversed, so that the apical bud is itself inhibited.

### Development of extra buds

From tubers of the Bliss Triumph variety seed pieces were prepared, each weighing about 25 gm. and bearing one eye. They were

<sup>1</sup> Published upon its recent receipt at the expense of the Boyce Thompson Institute for Plant Research.

soaked one hour in a 3 per cent aqueous solution of thiourea, were then rinsed in tap water, planted in soil, and stored in a cool place until sprouting began. The result is shown in fig. 1. It will be noted that out of twenty pieces treated, seventeen or eighteen show the development of more than one sprout per eye, and that in the case of the piece in the upper left hand corner eight sprouts had started.

It is true that not all buds which start will continue to grow, but figs. 2 and 3 show a later development of sprouts from a single eye

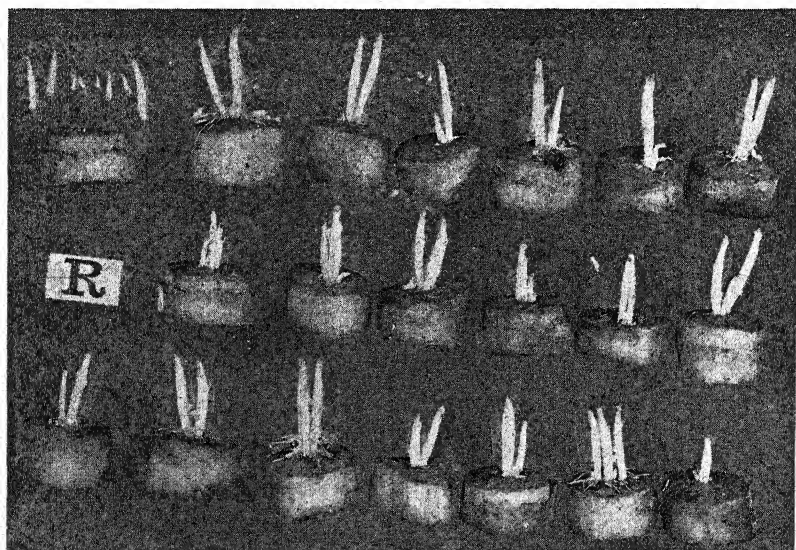


FIG. 1.—Bliss Triumph tubers soaked one hour in 3 per cent solution of thiourea; showing effect of treatment in causing development of more than one sprout per eye.

of another treated tuber. Fig. 3 shows five sprouts that grew from one eye, four of them at approximately the same rate; there is no indication here that one bud is influencing the growth of any other bud, although no doubt the growth of each was being restricted by the lack of sufficient available food in the mother piece. Fig. 3 also shows the development of side branches from a sprout in this early stage of development. With untreated tubers branching does not usually begin until a much later stage of growth. Branching in this case began as soon as the bud emerged from the tuber. It is possible that some of the extra sprouts may be branches from one sprout, but this did not appear to be the case when the cluster of sprouts

was separated. In any event it shows the effect of thiourea upon the course of development of the buds in the eye of the potato.

This multiple sprout effect was obtained with Bliss Triumph, Irish Cobbler, McCormick, and Rural New Yorker varieties. The concentration and time of treatment required to produce it varied with the variety and stage of dormancy, but a suitable range was found to be about 2-4 per cent thiourea, the soaking being continued for one to two hours.

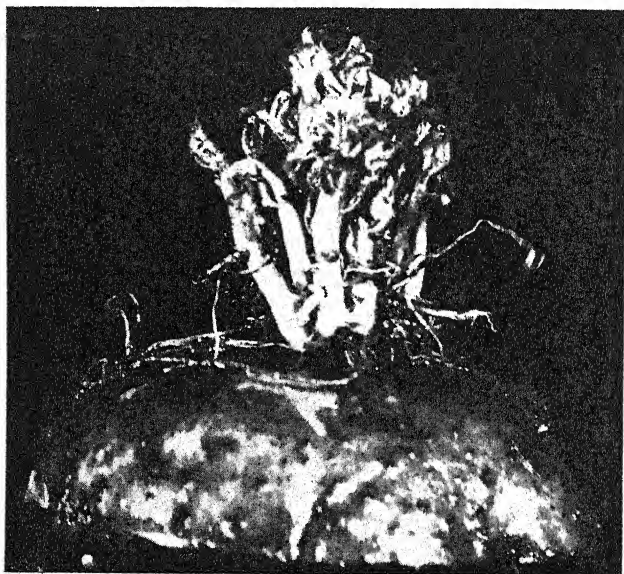


FIG. 2.—Effect of thiourea in inducing formation of several sprouts from one eye of potato (Bliss Triumph); cut tubers soaked one hour in 4 per cent thiourea.

More than 200 chemicals were tried, but thiourea was the only one that consistently forced multiple sprouts without causing rotting of the tubers. As shown in fig. 1, the surfaces of the treated pieces were bright and clear, indicating that no serious injury to the tissue had been caused. In many cases other chemicals caused the development of more than one bud per eye in a small percentage of the pieces treated; but this occurred when the concentration of chemical was high enough to cause considerable injury. In such cases many of the treated pieces rotted either wholly or partly.

The list of chemicals that did not give this multiple sprout response included representatives of various classes of compounds,

both inorganic and organic. Space is not available in this article to publish a complete list of chemicals used, but a few of them may be given, together with a description of the approximate range of concentration and time of treatment. The following were tried by the

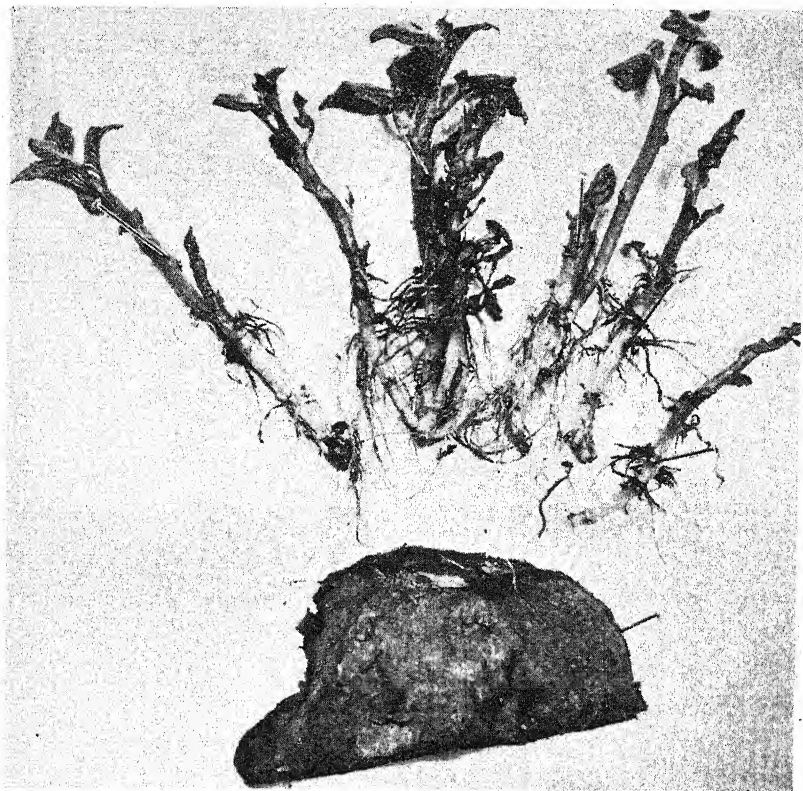


FIG. 3.—All sprouts shown grew from one eye of potato (Irish Cobbler); four out of five growing at approximately same rate; inhibition of growth of subsidiary buds by dominant bud prevented; cut tubers soaked one hour in 4 per cent thiourea.

soak method, the time of treatment being one hour:<sup>2</sup> acetamide 0.1-100 gm.; ethyl alcohol 0.3-9 cc. of 95 per cent; boric acid 0.25-10 gm.; bleaching powder (commercial) 2-80 gm.; chloral hydrate 0.25-10 gm.; ethylene glycol 1-25 cc.; ethylene oxide 0.1-10 cc.; furfural 0.5-20 cc.; pyridine 0.01-10 cc.; phenol 0.01-10 gm.; methyl-urea nitrate 1-10 gm.; mercuric chloride 0.1-1 gm.; manganous chloride

<sup>2</sup> The numbers placed after each chemical show the amounts per liter of water.

0.126-12.6 gm.; potassium permanganate 0.0156-15.6 gm.; sodium nitrate 0.85-59.5 gm.; potassium ferrocyanide 0.6-5 gm.; potassium ferricyanide 1-10 gm.; strontium nitrate 3-30 gm.; sodium potassium tartrate 3-30 gm.; ammonium sulphate 33-66 gm.

The following chemicals failed to induce the development of extra buds per eye by the vapor method, using cut tubers:<sup>3</sup> bromoform 0.02 for 3 hours to 0.3 cc. for 24 hours; chlorobenzene 0.06 cc. for 3-24 hours; ether 0.01-2 cc. for 3 hours; ethylene dibromide 0.001 cc. for 3 hours to 0.01 cc. for 24 hours; ethyl nitrite 0.01-0.3 cc. for 3 hours; gasoline 1 cc. for 1-24 hours; methylene chloride 0.5 cc. for 1-24 hours; picoline 0.01-0.3 cc. for 3 hours; acetylene tetrachloride 0.06-0.3 cc. for 16 hours; trimethylene chlorhydrin 0.1-0.5 cc. for 16 hours; toluol 0.06-0.3 cc. for 3 hours.

Injecting the tissue just below the bud with 1 per cent cane sugar and 3 per cent hydrogen peroxide did not force the sprouting of extra buds per eye; soaking in a complete mineral nutrient solution was also without effect.

A special test was made for the purpose of comparing the effect of thiourea and of other "urea" and "thio" compounds. Bliss Triumph dormant tubers from the 1925-26 crop from Bermuda were used. The potatoes were purchased in the Yonkers markets as soon as the Bermuda crop began to arrive. Because of the small size of some of the tubers the cut pieces did not all have only one eye each; some had two eyes. Table I shows the effect of the treatments upon the number of sprouts per seed piece that had appeared above ground after 35 days.

From table I it is seen that only thiourea showed any marked tendency to force the sprouting of more than one bud per seed piece. Urea was ineffective at the concentrations used, although it is possible that stronger percentages for longer periods of treatment would give better results. This should be tested. Ortho-tolyl-thiourea caused prompt sprouting of dormant buds, but double sprouts started from only one seed piece. Di-ortho-tolyl-thiourea was ineffective either in causing early sprouting or in forcing the growth of extra buds.

In these experiments the thiocyanates were next to thiourea in

<sup>3</sup> The numbers following each chemical show the amount of the chemical used per liter of air space in the container in which the treatment was carried out.



the order of effectiveness in respect to multiple sprout formation. In many experiments, not shown in table I, it was noted that double or triple sprouts from single eyes were not uncommon in treated lots with sodium, potassium, or ammonium thiocyanate. The thiocyanates also caused the development of sprouts that were noticeably plumper and fatter than normal sprouts (fig. 4). Occasionally these sprouts would also become twisted or gnarly. These results, although

TABLE I

EFFECT OF THIOUREA AND OF OTHER "THIO" AND "UREA" COMPOUNDS UPON  
NUMBER OF SPROUTS PRODUCED PER SEED PIECE OF POTATO

CHEMICAL	CONCENTRATION PER CENT	DURATION OF TREATMENT (HRS.)	NUMBER OF SPROUTS PER SEED PIECE	CHEMICAL	CONCENTRATION PER CENT	DURATION OF TREATMENT (HRS.)	NUMBER OF SPROUTS PER SEED PIECE
Thiourea.....	4	1	2.5	Allyl thiourea...	1	1	1.0
Thiourea.....	2	1	1.8	Phenyl thiourea.	*	1	0.5
Thiourea.....	4	1	3.7	Sodium thiocyanate.....	3	1	1.1
Thiourea.....	4	2	4.0	Ammonium thiocyanate.....	3	1	1.2
Thiourea.....	2	1	3.9	Sodium thiosulphate.....	8	1	0.1
Thiourea.....	2	2	3.9	Ammonium thiosulphate.....	8	1	Rotted
Urea.....	10	1	0.6	Potassium cyanide.....	0.1	1	0.5
Urea.....	8	1	0.1	Dicyandiamide..	3	1	0.0
Urea.....	2	1	0.3	Ethylenecyanide	0.2	1	0.3
Urea nitrate....	3	1	rotted	Check-H <sub>2</sub> O.....	.....	1	0.3
Urea nitrate....	1	1	0.2	Check-H <sub>2</sub> O.....	.....	2	0.9
Urea nitrate....	0.3	1	0.3				
O-tolylthiourea..	*	1	1.1				
O-tolylthiourea..	*	2	1.6				
Di-o-tolyl thiourea.....	*	1	0.2				
Di-o-tolyl thiourea.....	*	2	0.9				

\* Water-saturated solution.

frequently obtained, could not be produced at will, as was the case with the thiourea treatments, and therefore less emphasis is placed upon them.

#### Apical dominance

In order to determine the effect upon apical dominance, the tubers were cut lengthwise, into halves or quarters, depending on the size of the tubers. The cut pieces were then soaked in different concentrations of thiourea for different lengths of time, rinsed, and planted in soil and stored in a cool place. After sprouting began they were placed in a greenhouse.



The results of an experiment carried out in this way are shown in fig. 5. The checks in the bottom row show the normal behavior of Bliss Triumph tubers, three out of five pieces only showing one sprout per piece, and this sprout starting from the apical end. In this photograph the pieces are arranged so that the apical end is directed toward the right.

In the two treated lots (lots *G* and *K*, fig. 5), however, it will be noted that the apical end did not dominate the basal end. In most cases sprouts started from two or even more buds on each piece; in some cases buds started nearly as well from the basal end as from the apical. Several examples of multiple sprouts from single eyes are shown in the treated lots.

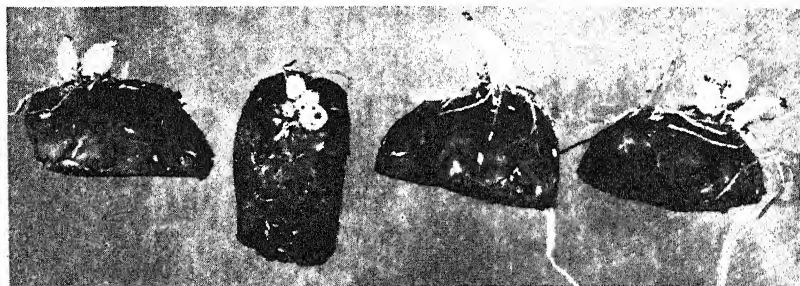


FIG. 4.—Effect of potassium thiocyanate in causing multiple sprouts at eye of potato (Bliss Triumph); note fatness of buds; this result frequently but not always obtained; 3 per cent potassium thiocyanate for one hour.

The behavior of Irish Cobbler and McCormick varieties toward thiourea treatments is shown in fig. 6. The treated lots (lots *M* and *Q*, fig. 6) show complete failure of apical dominance, and also numerous examples of multiple sprouts per eye. At the time the treated tubers were photographed the check tubers had not sprouted (lots *E* and *F*, fig. 6), and the figure therefore shows the condition of the checks six weeks after the picture of the treated lot was taken. The Irish Cobblers show the strong apical dominance of tubers of this variety. This dominance was less marked in the case of the McCormick variety, but the effect of the thiourea on the number of sprouts per eye is clearly shown.

In many cases it was found that the apical buds of tubers treated with thiourea did not grow, but that growth first started from buds

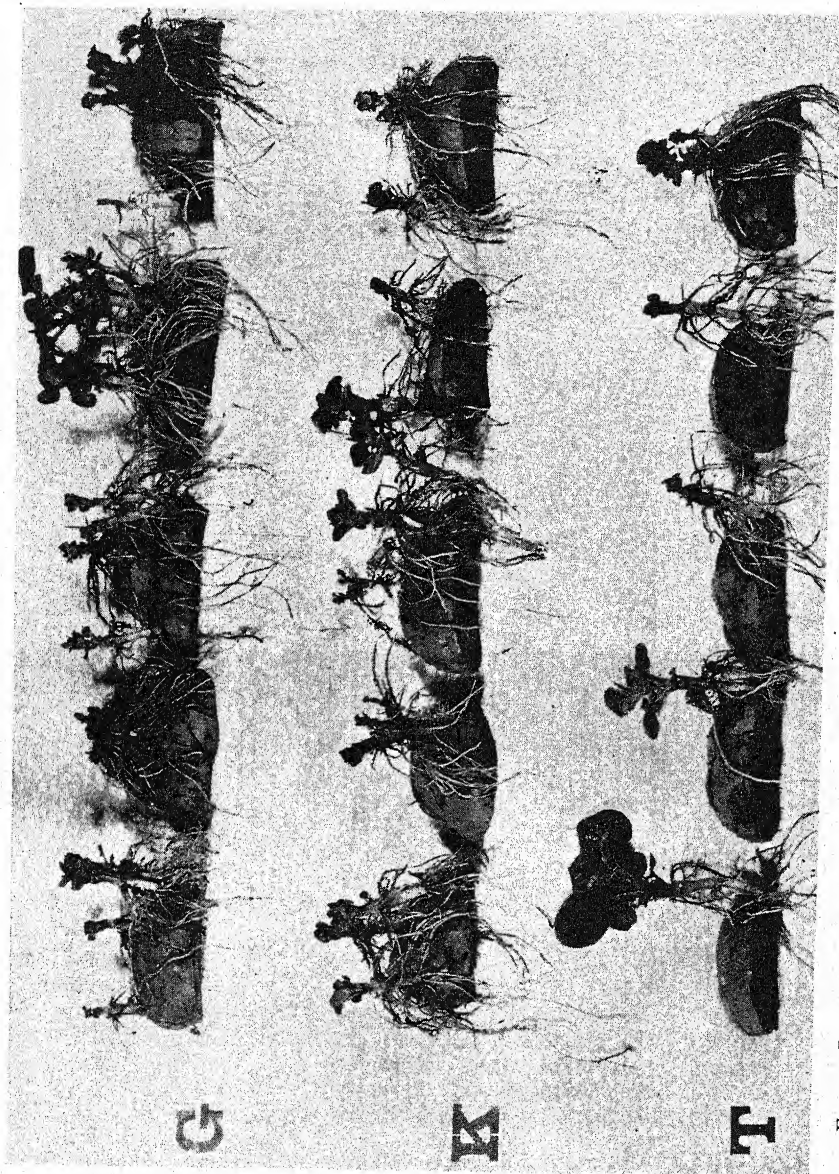


FIG. 5.—Lot G, tubers (Bliss Triumph) cut lengthwise into thirds and soaked two hours in 4 per cent thiourea; in each case apical end directed toward right; note absence of apical dominance and formation of multiple sprouts per eye. Lot K, same with 2 per cent thiourea for two hours. Lot T, check, soaked two hours in water; four out of five seed pieces show apical dominance, and three out of five show only one sprout per seed piece.

toward the basal end. This is shown in fig. 7 (top row, lot *D*), and the two pieces to the right in the second row (lot *H*). The failure

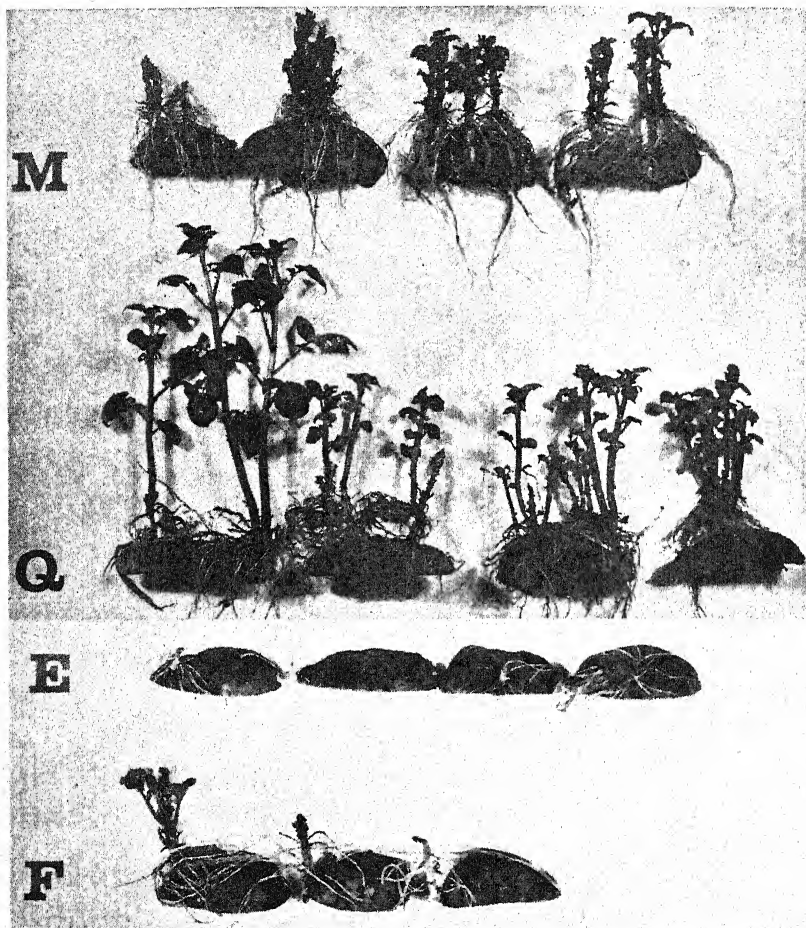


FIG. 6.—Lot *M*, tubers (Irish Cobbler) cut lengthwise into quarters, soaked two hours in 4 per cent thiourea; apical dominance disturbed and multiple sprouts per eye formed. Lot *Q*, same except McCormick variety used. Lot *E*, checks (McCormick) soaked two hours in water. Lot *F*, checks (Irish Cobbler) soaked two hours in water (lots *E* and *F* photographed 44 days after lots *M* and *Q*, but planted at same time).

of the apical buds to grow might have resulted from either of two causes: (1) the apical buds might have been injured or killed and

therefore incapable of growing; (2) the growth of the apical buds might have been inhibited by the growth of the basal buds that had started first.

That the first supposition is incorrect was proved by the fact that such apical buds, when cut off from the tuber and planted separately, sprouted at once. This is shown in fig. 7 (lot *K*, bottom row). The photograph was taken on the ninth day after the apical ends were removed and planted, showing that they had the capacity for prompt growth when removed from the inhibiting influence of the basal buds.

The results shown in fig. 7 indicate that by chemical treatment with thiourea the direction of dominance was reversed, a basally placed bud becoming dominant and inhibiting the growth of the apical bud. A reversal in the direction of dominance was also reported by CHILD and BELLAMY (4) for *Phaseolus*. They found that non-apical buds, forced into growth as a result of physiological isolation from the tip, "in some cases may even inhibit the further growth of the chief tip." The situation here would be analogous if we assume that the thiourea treatment had caused physiological isolation of the apical buds. In what manner this isolation could be brought about by the treatment, however, is not known.

### Discussion

The question whether this is a specific response to thiourea cannot be answered by these experiments. It can merely be stated that out of a list of more than 200 chemicals, representing various classes of chemical compounds, thiourea alone showed this effect upon tubers of these varieties. Further experiments would be necessary to determine whether by proper choice of concentration and time of treatment other chemicals could cause similar responses. While it seems unlikely that this is a specific response, it would be desirable to examine the possibility more closely; for if the number of chemicals that can influence bud dominance in this manner can be restricted to one, or only a few, or even to a certain group or type, the study of the cause of dominance will be much simplified.

APPLEMAN (2) expressed the opinion that any treatment that weakened the sprouts destroyed the dominance of the apical bud.

Thus successive removal of sprouts and certain storage conditions resulted in loss of apical dominance. It is true that tubers treated

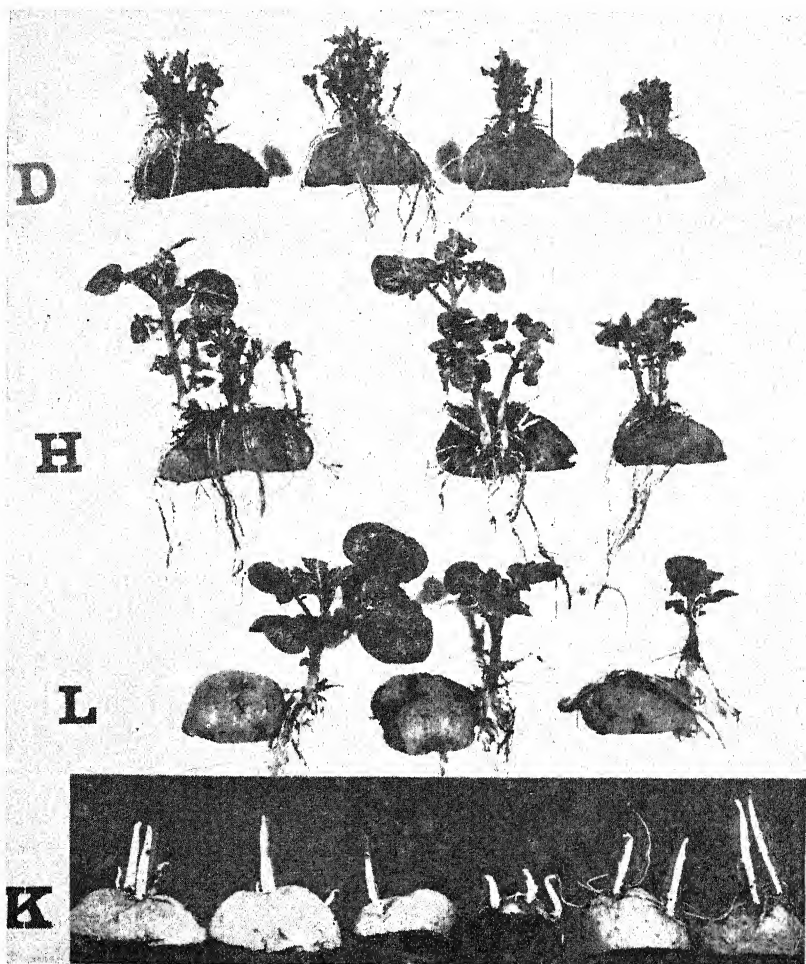


FIG. 7.—Lot *D*, tubers (Bliss Triumph) cut lengthwise into quarters, soaked one hour in 4 per cent thiourea; apical ends directed toward right; note apical buds were not growing, but buds more basally placed started into growth first. Lot *H*, same with 2 per cent thiourea for two hours; apical buds in the two pieces at right not growing. Lot *L*, checks, soaked two hours in water; only apical end growing and only one bud per eye started growth (compare lots *L* and *D*). Lot *K*, apical ends cut off from tubers like those shown in lot *D* and planted separately; inhibited apical buds started growth at once (photograph of lot *K* taken 9 days after planting).

with thiourea under conditions that forced many sprouts per eye did not give strong sprouts. They were thin and spindly. When six or seven were produced from one eye the sprouts were hardly stiff enough to stand upright. This may not be an expression of weakness, however; it may merely mean that there was insufficient food or other growth-promoting substances in the mother tuber to support so many sprouts.

From the standpoint of toxicity it is certain that thiourea is less toxic to potato than many of the substances used. Rotting of the treated pieces was common with most of the chemical treatments, but the thiourea treated lots were notably free from rot. Fig. 1 shows that the surfaces of the treated pieces were clean and free from discoloration or any other evidence of injury. Even when the treatment was severe enough to produce the result shown in fig. 2, the potato tissue was perfectly sound.

It is clear, therefore, that if this disturbance of the normal system of bud correlation in the potato is to be ascribed to a toxic effect, the injury must be caused in some special manner in the case of thiourea. It is believed that factors other than mere injury to the tissue are involved.

At least three theories have been offered to explain why the apical bud is able to prevent the growth of basal buds: (1) that the apical bud produces an inhibiting substance which travels in a basipetal direction and prevents the growth of buds lower down on the stem (ERRERA 6, REED and HALMA 10); (2) that there is a higher state of metabolic activity at the tip, and that, as a result of the electric potential set up by this physiological gradient, control is transmitted by way of the protoplasm and in some manner represses the growth of sub-apical buds, but not by the passage of any material substances (CHILD 5, CHILD and BELLAMY 4); (3) that not enough nutritive materials are available for all the buds present and that the growing tip causes a flow of materials toward itself and away from the basal buds (GOEBEL 8, GARDNER 7). Theories 1 and 3 are also fully discussed by McCALLUM (9).

If inhibition is produced by a substance that is formed by the apical bud, the thiourea effect could be explained on the assumption that the treatment either prevents the apical bud from forming the



substance, or inactivates it in some way after it is formed. The fact that thiourea can nullify this inhibiting effect under certain definite conditions should be of great assistance in experimental work relating to the nature and mode of action of this hypothetical inhibitory chemical.

If the second supposition is correct, then the thiourea may operate, either by preventing the transmission of the stimulus, or by changing the relative metabolic activity of the apical and subapical buds. The results of the experiments, indeed, do show an increase in the rate of growth and therefore of metabolism in the basal buds, but there is little evidence yet to explain how thiourea can produce this change; and there is less to show how thiourea can cause the protoplasm to lose the capacity of transmitting the inhibitive stimulus.

There may be some evidence in favor of the third view, in that thiourea may contribute additional nutrient material by means of which additional buds may develop; but other nutritive substances were not able to induce this response. We would be compelled, therefore, to assume that additional amounts of only certain nutritive substances are necessary to initiate a renewal of growth in the inactive buds. We do not yet know how far the thiourea penetrates into the potato, nor what changes it may itself undergo after entrance into the tissue.

### Practical considerations

When the forcing of multiple sprouts by solutions of thiourea was first noted, it was thought that from a practical standpoint this would be an unfavorable response; additional sprouts per seed piece would result in the formation of many small tubers per hill. It was pointed out to the writer by WILLIAM STUART of the United States Department of Agriculture, however, that when potatoes are produced for seed purposes and not for table use, a large proportion of moderately small tubers is desirable, since this size gives greater economy in planting a crop. A chemical treatment that would regulate with security the number of sprouts per hill, therefore, might find application. A further use for such a treatment might be found in the case of varieties that send out too few sprouts per seed piece.

The present high price of thiourea, however, about \$15.00 per kilogram, would likely preclude its use in a practical way, and the possibility of using this chemical for such purposes will depend upon finding a cheaper supply of it. It may be that the high price is caused by the methods of purification, and that the impure or unrefined chemical would give equally good results in the potato treatments.

### Summary

1. Solutions of thiourea ( $\text{NH}_2\text{CSNH}_2$ ) caused the growth of two or more (often four or five) buds from a single eye of the potato.

2. This result was not caused by any other chemical at the concentrations and periods of treatment tried, although more than 200 chemicals representing various classes of chemical compounds were tested.

3. Several "urea" and "thio" compounds were tried, but none showed the consistent results that were obtained with thiourea. Next to thiourea, the most favorable chemicals for this purpose were the thiocyanates.

4. Solutions of thiourea also prevented the apical buds of the tubers from completely inhibiting the growth of basal buds. Certain cases were found in which the direction of dominance was reversed so that the apical buds themselves were inhibited. Such inhibited apical buds when cut off from the tuber and planted separately started into growth at once, after being removed from the influence of previously sprouted basal buds.

5. The relation of these facts to certain theories regarding the cause of the inhibition of basal buds by tip buds is discussed.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH  
YONKERS, N.Y.

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## SECOND REPORT ON THE USE OF CHEMICALS FOR HASTENING THE SPROUTING OF DORMANT POTATO TUBERS<sup>1</sup>

F. E. DENNY

(Received for publication April 9, 1926)

### INTRODUCTION

In a previous paper (1) it was shown that a number of different chemical treatments were effective in breaking the rest period of Irish potatoes (*Solanum tuberosum* L.). In the autumn and winter of 1925-1926 a large number of additional tests were carried out, using the same chemicals and also others not previously tried. This paper gives the results of these later tests, and offers suggestions as to the most promising methods of treatment based on the experience so far obtained with the different varieties.

### VARIETIES USED

The Bliss Triumph potatoes were from the Maine 1925 crop and were shipped by express at once after digging. They were fully mature but dormant. Untreated tubers planted October 15 showed general sprouting above ground about December 5.

The Irish Cobbler potatoes were from the late crop in New Jersey. They were harvested October 26 from vines that were still green when caught by frost. They were therefore immature when dug and were very dormant. General sprouting of untreated tubers did not occur until February 15, 1926. Since this lot of Irish Cobblers was immature when dug, a test was made using the same treatment at three different dates, namely, 10 days, 22 days, and 36 days after digging. The results obtained depended partly upon the time after digging at which the treatment was applied.

Potatoes of the Green Mountain variety were obtained from the late crop in Western Maryland. They were shipped by express to Yonkers, and treatments were started within about one week after the tubers had been removed from the soil. These tubers were not very dormant, untreated tubers showing good sprouting in about two months after planting.

The McCormick potatoes were grown in the Institute gardens as a late crop, were harvested October 15, and stored at room temperature. Experimental work with McCormick was not begun until November 10. In considering the results with McCormick, therefore, it should be noted that

<sup>1</sup> Published, at the expense of the Boyce Thompson Institute for Plant Research, out of the order determined by the date of receipt of the manuscript.

the tubers were not treated until nearly a month after digging. They were deeply dormant, however, the untreated tubers not showing general sprouting until February 15, 1926.

### METHODS

Three methods of treating the tubers were used. They will be referred to in the following pages as the "dip method," "vapor method," and "soak method." The general procedure with each method (omitting, for the present, consideration of the particular concentration of chemical and time of treatment) was as follows:

*Dip Method:* The tubers were cut into suitable size for planting (about 1 oz.), were dipped into a solution made by dissolving a certain amount of chemical in water, were removed at once from the dipping solution, and were placed in a closed container that had a volume not more than about twice the volume of the sample, and were stored in the container for a certain length of time. This method could be used with substances like ethylene chlorhydrin, which give off a vapor on standing. By this procedure a uniform distribution of liquid over the surface of the potato was obtained. The vapors penetrated the tissue and brought about changes which resulted in early sprouting. The concentrations and period of storage will be discussed when consideration is given to the results with the different varieties.

*Vapor Method:* Either whole tubers or cut tubers ready for planting were placed in metal containers that could be closed (in ash cans, for example). The desired amount of volatile chemical was placed in shallow dishes that stood on pedestals inserted for the purpose of raising the chemical above the potatoes in order to favor evaporation. The air was not stirred, except in some of the later experiments in which a special gas-tight house of about 150 cubic feet capacity equipped with an electrical fan was used. For large-scale treatments it would probably be necessary to stir the air with fans in order to obtain an even distribution of the vapors which, in general, are heavier than air.

*Soak Method:* The cut tubers were soaked in solutions of different chemicals of varying concentrations for periods of time varying from one to two hours. After the period of treatment the treated pieces were rinsed in tap water and planted. The soak method has two serious disadvantages: (1) the trouble involved in handling potatoes when the treated material must remain submersed for an hour or two, and (2) the diluting effect on the soaking solution that results from successive treatments in the same solution. Nevertheless, this method gave good results especially when thiocyanate was used with Irish Cobbler.

## EXPERIMENTAL

Results with Ethylene Chlorhydrin ( $\text{C}_2\text{H}_4\text{Cl}_2$ )<sup>2</sup>

*Dip Method:* The dip method, using a dipping solution containing concentrations of ethylene chlorhydrin varying from 15 cc. to 45 cc. per liter of water and with subsequent storage periods varying from 16 to 24 hours, or with a dipping solution of 5 to 20 cc. per liter with a storage period of 48 hours, gave notably good results with Bliss Triumph. Photographs of these results are shown in Plate XXXI, figures 1 and 2, and Plate XXXII, figures 4 and 5. It is apparent from the photographs that there is a considerable range of concentration of dipping solution and of time of subsequent storage over which forcing effects can be obtained; but it is also seen that within this range a maximum effect is attainable. No doubt the conditions necessary for a maximum will be found to vary with the stage of dormancy and possibly also with the crop from different localities or with the harvest of different years. Whether it is possible to find a treatment that will permit of the use of the most favorable concentration and time of treatment under any given set of conditions can be determined only by further experiments.

The dip method also gave good results with the Green Mountain variety, using the same concentrations and same lengths of exposure as those shown above for Bliss Triumph. In the case of the McCormick variety, cut tubers, the dip method, using 30 cc. and 45 cc. per liter, and storing 16 hours, gave good stimulation as is shown in Plate XXXII, figure 6, lots B and C. This appeared to be the minimum strength, however, and it may be that the storage period should be increased for McCormick. Cut tubers of the Irish Cobbler variety when treated 10 days after digging did not respond to the dip method at these concentrations and for storage periods up to 24 hours; better results were obtained by treating 22 days after digging, but it was not until 36 days after digging that good forcing effects were obtained. Further experiments will be carried out, using longer storage periods after dipping.

*Vapor Method:* The vapor method, using cut tubers of the Bliss Triumph variety, gave good forcing action at a concentration of 0.5 cc. per liter of air space for 24 hours and 1.0 cc. per liter of air space for 16 hours; but 1 cc. for 24 hours injured, and 0.25 cc. for 48 hours, although the results were better than those with the check lot, showed less favorable stimulation. The sprouting of Green Mountain tubers was hastened by 0.5 cc. ethylene chlorhydrin per liter of air space for 16 hours, and the same concentration for 24 hours was satisfactory for McCormick. In the case of the Irish Cobbler variety, the vapor method, using 0.5 cc. per liter of air space for 16 hours, hastened sprouting at the 22-day period, but was ineffective at the 10-day period and caused some injury at the 36-day period after digging.

<sup>2</sup> The ethylene chlorhydrin referred to in this paper is the commercial 40-percent solution, not the anhydrous chemical.

One experiment was carried out with Bliss Triumph, using the vapor method with whole tubers and planting the intact tuber without cutting it into pieces. In this case 2 cc. of ethylene chlorhydrin per liter of air space were used, the period of treatment being 24 hours. Favorable results were obtained, as are shown in Plate XXXIII, figure 7. In considering this photograph it should be noted that, while the check tubers sent out one sprout per tuber, the treated potatoes sent out several sprouts per tuber, all sprouts being located at the apical end. The sprouts were vigorous in spite of their number.

When whole tubers were treated by the vapor method and planted cut, the result obtained depended upon whether the tubers were cut and planted at once or were stored in air for various periods before cutting and planting. It was found that storage after treatment, before planting, avoided toxicity and that the favorable effects of the treatment in hastening germination were not lost by delaying the time after treatment at which the tubers were cut into pieces and planted in the soil. This phase of the work is discussed in another section of this paper (p. 391).

*Soak Method:* Experiments with Bliss Triumph showed that soaking one hour in a solution containing 4 to 6 cc. of ethylene chlorhydrin per liter of water gave good forcing action. However, when results can be obtained by either the dip method or the vapor method (as is the case with Bliss Triumph) the soak method is not recommended. In the case of McCormick potatoes, 4 cc. per liter and soaking 2 hours gave hastened germination (Pl. XXXIII, fig. 9, lot U), and 6 cc. per liter forced the Irish Cobblers even at the 10-day period after digging. This treatment was also satisfactory at the 22-day period but caused injury at the 36-day period.

### Results with Solutions of Sodium and Ammonium Thiocyanate (NaCNS), (NH<sub>4</sub>CNS)

*Bliss Triumph:* Soaking cut tubers one hour in 2-percent sodium thiocyanate caused a little injury but forced early sprouting of those not injured. One-percent sodium thiocyanate for 1 hour or for 2 hours was favorable, and sprouting considerably better than that of the untreated lots was obtained with 0.5 percent for 2 hours. In a subsequent experiment, using Bliss Triumph from Bermuda, the results with ammonium thiocyanate were better than with sodium thiocyanate, good results being obtained with 3-percent, 2-percent, or 1-percent ammonium thiocyanate for 1 hour. The margin between stimulative and injurious concentrations has not been large in the case of sodium or potassium thiocyanate treatments with Bliss Triumph. It may be that ammonium thiocyanate will give a wider range. Where a reduction in the percentage germination would not be serious, for example, in forcing early germination of tubers for experiments in plant pathology, the thiocyanate treatment could be used with confidence; but for general planting, where a full stand is wanted, the thiocyanate treatment has not yet given a good margin of safety for Bliss Triumph.

*Irish Cobbler:* In the case of Irish Cobbler, however, the thiocyanate treatment has been uniformly successful. The soak method, using 2-percent sodium thiocyanate for 1 hour, was notably successful with this lot of Irish Cobblers at all three periods of treatments. Four-percent sodium thiocyanate broke the dormancy at the 10-day period, but caused injury at the 22-day period. One-percent sodium thiocyanate was partly effective at the 10-day period after digging and gave excellent stimulation at the 22- and 36-day periods. Plate XXXIII, figure 8, shows the results obtained with sodium thiocyanate.

*Green Mountain:* Early sprouting of this variety was forced by soaking 1 hour in 2-percent sodium thiocyanate.

*McCormick:* Soaking cut tubers for 1 hour in 2-percent sodium thiocyanate and for 2 hours in 1-percent sodium thiocyanate gave good sprouting, as is shown in Plate XXXIII, figure 9, lots *T* and *V*. Four-percent sodium thiocyanate for 1 hour was too strong.

### Results with Ethylene Dichlorid ( $C_2H_4Cl_2$ )

*Bliss Triumph:* Ethylene dichlorid, a chemical first recommended by McCallum (2), gave promising results. Cut tubers responded well to a 24-hour treatment with a concentration equal to 0.5 to 1.0 cc. of ethylene dichlorid in 17.5 liters of air space (equal to 0.028 to 0.057 cc. per liter of air). An injurious concentration for Bliss Triumph was reached at about 2.0 cc. for a 17.5-liter space (about 0.1 cc. ethylene dichlorid per liter of air).

*McCormick:* A favorable response of the McCormick variety to vapors of ethylene dichlorid was obtained, as is shown in Plate XXXI, figure 3, lots *J*, *K*, and *L*. The effective concentration ranged from 2.0 cc. to 0.5 cc. in 17.5 liters of space (from 0.1 to 0.028 cc. per liter of air). For whole-tuber treatments, 4 cc. in 17.5 liters of air (0.2 cc. ethylene dichlorid per liter of air) for 24 hours caused early sprouting. Half the concentration was noticeably less effective. It will be noted that relatively low concentrations of ethylene dichlorid were effective. Because of its cheapness and availability as a standard commercial product, it is hoped that a favorable procedure can be worked out.

### Results with Ethyl Bromid ( $C_2H_5Br$ )

Ethyl bromid, another chemical suggested by McCallum (2), forced the sprouting of Bliss Triumph, cut tubers, by a 48-hour treatment, using 0.5 cc. for each 17.5 liters of air space (0.028 cc. per liter of air), and fairly well by a 24-hour treatment, using 1.0 cc. for each 17.5 liters of air space.

In the case of the McCormick variety, ethyl bromid was successfully used with cut tubers at a concentration of 3 cc. in a 17.5-liter space for 16 hours (see Pl. XXXI, fig. 3, lot *M*). With whole tubers specially favorable results were obtained with 4 cc. in a 17.5-liter space for 24 hours.



### Results with Thiourea ( $\text{NH}_2\text{CSNH}_2$ )

Soaking cut tubers in 2- to 4-percent thiourea solutions broke the dormant period of Bliss Triumph, Irish Cobbler, and McCormick varieties. Several derivatives of thiourea were tested, and one of these, o-tolylthiourea, gave good results, a saturated solution of this chemical causing early germination after 1 hour's soaking.

Thiourea also caused the development of more than one sprout per eye, and in some cases more than one eye per seed-piece. This effect upon bud-inhibition and apical dominance will be considered more fully in another paper (Bot. Gaz., in press).

### Results with Other Chemicals

Ethyl iodid,  $\text{C}_2\text{H}_5\text{I}$ , a substance not heretofore used for breaking the dormancy of potatoes but tested by Stuart (3) on dormant bulbs and shrubs, was effective with cut tubers of the Bliss Triumph variety in unusually low concentrations,  $\frac{1}{6}$  cc. in a 17.5-liter space for 24 hours giving good sprouting (equal to 0.0095 cc. per liter of air).

Xylol,  $\text{C}_6\text{H}_4(\text{CH}_3)_2$ , hastened sprouting fairly well when 1 cc. in a 17.5-liter space was applied to cut tubers for 24 hours. The range with carbon tetrachlorid,  $\text{CCl}_4$ , was very narrow, 2 cc. in a 17.5-liter space being too high and 0.5 cc. being too low. When 1.0 cc. was used the results were satisfactory. Dichloroethylene,  $\text{C}_2\text{H}_2\text{Cl}_2$ , trichloroethylene,  $\text{C}_2\text{HCl}_3$ , and carbon bisulphid,  $\text{CS}_2$ , gave results similar to those reported in the previous paper (1).

Ethylene,  $\text{C}_2\text{H}_4$ , propylene,  $\text{C}_3\text{H}_6$ , and acetylene,  $\text{C}_2\text{H}_2$ , were tried with both whole and cut tubers at concentrations of 1 to 100 and 1 to 1,000 of air for 4 days and 7 days. In no case were favorable forcing effects noted. Methyl chlorid gas at a concentration of 1 part methyl chlorid to 100 parts of air, for 4 days, gave considerably better responses. The concentration and time of treatment required for this gas, however, make its use impractical.

Ethylene dibromid,  $\text{C}_2\text{H}_4\text{Br}_2$ , was notable, not for its forcing effects but because of its high toxicity. Even 0.01 cc. in 17.5 liters of air space injured cut tubers of Bliss Triumph with a 24-hour treatment. This is equivalent to about 0.0006 cc. of ethylene dibromid per liter of air space. The stimulative effects of this chemical, however, at any concentration tried were slight or negligible.

### Effect of Storage in Air after Treatment on the Toxicity of Chemicals and upon Subsequent Sprouting of Tubers

When it was found that whole tubers could be successfully treated with vapors, the question at once arose, Must the treated tuber be planted at once, or can it remain in storage until a later planting time without losing the benefit of the treatment? It was found not only that storage subsequent to treatment was possible, but that the toxic effects of the vapor were wholly or at least partly avoided.

Thus, Bliss Triumph whole tubers were treated with concentrations of ethylene chlorhydrin varying from 6 cc. per liter of air space for 24 hours to 1 cc. for 48 hours. A few tubers were removed at once, were cut into pieces, and planted. The remainder of the treated tubers were stored in air in paper bags, and a sample was removed at intervals of one week and planted after being cut into pieces in the usual way. The tubers that were cut and planted at once after treatment rotted, but those planted after subsequent storage in air did not rot but sprouted much sooner than check tubers, handled in the same way but receiving no chemical treatment. Thus, as appears in Plate XXXIV, fig. 11, the treated lot after storage in air for 7 days showed the favorable effect of the chemical treatment, and in Plate XXXIV, figure 12, it is seen that the forcing effect of ethylene chlorhydrin is not lost by storage in air for 21 days after treatment. In fact, in the experiment, the treated tubers began to sprout in air before they were planted, which fact indicates that the growth processes that are started by the treatment continue in action without requiring the favorable influence of a moist soil.

The same effect to a less marked extent was noted with Irish Cobbler. Whole tubers treated with 1 cc. of ethylene chlorhydrin for 24 hours when cut into pieces and planted at once after treatment rotted. But when stored 7 days in air after treatment before cutting and planting, 9 out of 14 pieces lived and sprouted, and after storage for 14 days 13 out of 14 pieces lived and sprouted.

In the case of the Green Mountain variety, 4 cc. of ethylene chlorhydrin per liter of air for 24 hours killed the tubers if they were cut and planted at once after treatment; but, if the whole tubers were allowed to stand in air for 4 days, prompt and complete germination resulted. When the concentration used was 1 cc. per liter for 24 hours, 50 percent loss resulted when the tubers were cut and planted at once; but only 1 day's standing in air before planting was required to prevent this toxicity and at the same time to hasten the sprouting of the buds.

The fact that potatoes may be treated previous to the time at which they are to be planted is important from a practical standpoint, for it indicates the possibility that the tuber treatments could be made in large quantities by firms that grow potatoes for seed. The treated potatoes could then be shipped to purchasers and would arrive ready for immediate planting. Additional experiments are needed before such a procedure can be recommended. It must be shown, first of all, that tubers of various varieties can be successfully treated at once after removal from the soil, and secondly, that uniform results with the crops from different soils and in different years can be obtained.



## Results in Special "Gassing-house" under Semi-commercial Conditions

A special gas-tight room with a volume of 150 cubic feet was constructed; it was equipped with an electric fan for stirring the air, and had removable shelves with wire-screen bottoms for holding the potatoes.<sup>3</sup> From preliminary experiments carried out, using this room, it is likely that a smaller amount of ethylene chlorhydrin can be used under these conditions than was found necessary when the air was not stirred. Thus, McCormick whole tubers responded to a concentration of 0.35 cc. per liter of air space for a 20-hour treatment (see Pl. XXXIV, fig. 10). Much larger amounts were required under conditions in which the air was not stirred. This result is probably due to the fact that a more complete evaporation of the ethylene chlorhydrin was obtained by stirring the air. Since the ethylene chlorhydrin is a 40-percent solution in water, the vapors are not completely removed from the water unless the air is stirred.

### Comparison with Results of Previous Experiments

In general the results previously reported were confirmed, the same chemicals giving good forcing action on dormant tubers. The principal differences between the results of the first series of experiments and those reported in this paper are: (1) The margin of concentration for the soak method with Bliss Triumph was given as 3 to 10 cc. of ethylene chlorhydrin per liter of water. In the second series not more than 6 cc. per liter could be used without injury. (2) The ethylene chlorhydrin treatments were less favorable with Irish Cobbler in the second series. Irish Cobbler potatoes treated at once after removal from the soil did not respond satisfactorily to this treatment; after a storage period of about 22-36 days the treatments showed good results. It may be that the immaturity of this lot of Irish Cobblers as compared with those of the 1925 experiments may account for the difference. Further experiments on this point will be carried out.

### RECOMMENDED TREATMENTS

I list below the treatments that have been found successful with different varieties in these tests. Since the problem is still in the experimental stage, and since potatoes grown in different localities and in different stages of dormancy may respond differently, a range of concentrations and times of treatment is given in order that other experimenters may more easily determine the most favorable treatment for their special conditions.

*For Bliss Triumph:* Ethylene chlorhydrin, dip method, cut tubers, 30 cc. per liter of water, storage time 24 hours; or 40 cc. for 16 hours; or 10 cc. for 48 hours; ethylene chlorhydrin, vapor method, cut tubers, 0.5 cc. per liter of air space for 24 hours; or 1 cc. per liter of air space for 16 hours. Ethylene chlorhydrin, whole tubers, 1 cc. per liter of air space, for

<sup>3</sup> I am indebted to Mr. William Stuart of the United States Department of Agriculture for suggestions regarding the construction of this room.

24 hours; let the whole tuber stand in air for one or two days after treatment, then cut into pieces and plant. Ammonium, sodium, or potassium thiocyanate, 2-percent solution, soak for 1 hour; also 3-percent for 1 hour, or 1-percent for 2 hours; the tubers must be dormant for this treatment, otherwise injury will be likely to be produced.

*For Irish Cobbler:* Ammonium, sodium, or potassium thiocyanate, cut tubers, 2-percent solution, soak for 1 hour; also 3-percent for 1 hour, or 1-percent for 2 hours. Ethylene chlorhydrin, cut tubers, soak method, 6 cc. per liter of water, 1 hour; also 4 cc. per liter of water for 1 hour or for 2 hours. With tubers that were mature at the time of harvest the following treatment gave good results: ethylene chlorhydrin, vapor method, whole tubers, 0.5 cc. per liter of air space for 24 hours, tubers cut into pieces after treatment and planted.

*For Green Mountain:* Same as for Bliss Triumph.

*For McCormick:* The recommendations are based upon treatments applied to tubers that had been stored about a month in air after digging. Ethylene chlorhydrin, dip method, 45 cc. per liter of water, store 24 hours; or 30 cc., store 24 hours; or 20 cc., store 48 hours. Ethylene chlorhydrin, vapor method, cut tubers, 0.5 cc. per liter of air space for 24 hours; or 1.0 cc. for 16 hours, or 0.25 cc. for 24 hours. Ammonium, sodium, or potassium thiocyanate, cut tubers, 1 hour in 2- or 3-percent solution; or 1 hour or 2 hours in 1-percent solution. Ethylene dichlorid, cut tubers, 0.06 cc. per liter of air space for 16 hours; also 0.1 cc. or 0.03 cc. for 16 hours. Ethylene dichlorid, whole tubers, 0.2 cc. per liter of air space for 24 hours.

#### SUMMARY

1. In general, the results of former experiments were confirmed. Ethylene chlorhydrin, sodium thiocyanate, ethylene dichlorid, ethyl bromid, carbon bisulphid, and trichloroethylene forced early sprouting of dormant potatoes.

2. The principal difference found in the second series of experiments as compared with the first was that ethylene chlorhydrin was less effective against Irish Cobbler in the early stages of dormancy. This difference may be due to the immaturity of the tubers of this lot at the time of digging.

3. The following chemicals not mentioned in the previous paper were found to be effective in hastening sprouting: ammonium thiocyanate, ethyl iodid, and o-tolyl-thiourea.

4. Four varieties were tested: Bliss Triumph, Irish Cobbler, Green Mountain, and McCormick. Recommendations based on experience obtained in both series of tests are given for forcing the sprouting of tubers of these varieties.

5. It was found that, after whole tubers had been treated by the vapor method, it was not necessary to plant the treated tubers at once. The favorable effects remained in the tuber for at least three weeks after treat-

ment; in fact, the tubers began to sprout in air before being planted in the soil. This indicates the possibility that tubers could be treated in the locality where they are grown, and shipped to a distant locality for planting.

6. Storage of whole tubers in air after treatment before planting also aided in avoiding the toxicity of the chemicals. Tubers treated with certain concentrations of ethylene chlorhydrin, when cut into pieces and planted at once, often rotted, but samples of the same lot held in air a few days after treatment before cutting and planting did not rot, but showed early sprouting with healthy sprouts.

7. The effect of solutions of thiourea in causing the sprouting of multiple buds per eye, and in partially nullifying the dominance of the apical bud, was again noted in treatments with the Bliss Triumph, Irish Cobbler, and McCormick varieties.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.,  
YONKERS, NEW YORK

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#### EXPLANATION OF PLATES

##### PLATE XXXI

FIG. 1. Lot *T*: Ethylene chlorhydrin, dip method, cut tubers; used dipping solution containing 10 cc. per liter of water; storage period 24 hours. Bliss Triumph. Lot *V*: Same, but used 20 cc. of ethylene chlorhydrin per liter of water.

FIG. 2. Lot *W*: Same, but used 40 cc. of ethylene chlorhydrin per liter of water. Lot *X*: Check, same, but used water instead of ethylene chlorhydrin.

FIG. 3. Lot *J*: Ethylene dichlorid, vapor method, cut tubers; 2 cc. in 17.5 liters of air space for 16 hours. McCormick. Lot *K*: Same, but used 1 cc. Lot *L*: Same, but used 0.5 cc. Lot *M*: Ethyl bromid, vapor method, cut tubers; 3 cc. in 17.5 liters of air space for 16 hours. McCormick. Lot *N*: Check, cut tubers stored in air in closed container for 16 hours, then planted. Lot *O*: Same, but stored 7 days. Lot *P*: Same, but stored 4 days. *Note*: Lots *O* and *P* were checks on a treatment given to other lots not shown in figure 3.

##### PLATE XXXII

FIG. 4. Lot *Z*: Ethylene chlorhydrin, dip method, cut tubers; used dipping solution containing 5 cc. of ethylene chlorhydrin per liter of water; storage period 48 hours. Bliss Triumph. Lot *M*: Same, but used 10 cc. of ethylene chlorhydrin per liter of water.

FIG. 5. Lot *P*: Same, but used 20 cc. of ethylene chlorhydrin per liter of water. Lot *Q*: Check, same, but used water instead of ethylene chlorhydrin.

FIG. 6. Lot *A*: Ethylene chlorhydrin, vapor method, cut tubers; 0.5 cc. per liter of air space for 16 hours. McCormick. Lot *B*: Ethylene chlorhydrin, dip method, cut tubers; dipping solution 45 cc. of ethylene chlorhydrin per liter of water; storage period 16 hours. McCormick. Lot *C*: Same, but 30 cc. of ethylene chlorhydrin per liter of water. Lots *D* and *E*: Checks, not treated. *Note*: Lot *W*, shown in figure 9, Plate XXXIII, is also a check on lot *A* shown in this figure.

## PLATE XXXIII

FIG. 7. Lot *E*: Ethylene chlorhydrin, vapor method; tubers treated whole and planted intact; 2 cc. of ethylene chlorhydrin per liter of air space for 24 hours. Bliss Triumph. Lot *M*: Checks, not treated.

FIG. 8. Lot *F*: Sodium thiocyanate, soak method, cut tubers; 4 percent for 1 hour. Irish Cobbler. Lot *G*: Same, but used 2 percent sodium thiocyanate. Lot *H*: Checks, soaked 1 hour in water.

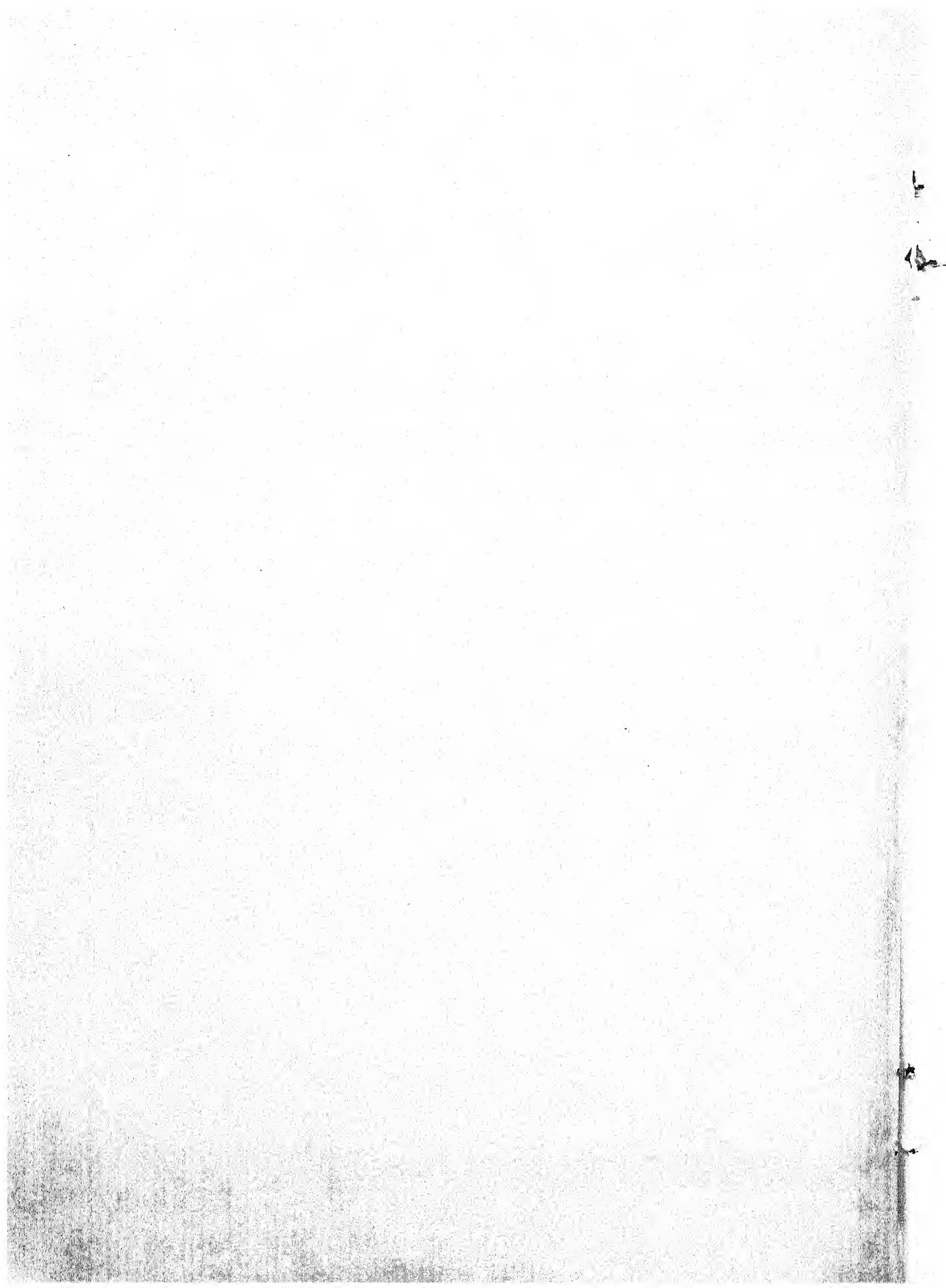
FIG. 9. Lot *T*: Sodium thiocyanate, soak method, cut tubers; 1 percent for 2 hours. McCormick. Lot *V*: Same, but 2 percent, 1 hour. Lot *U*: Ethylene chlorhydrin, soak method, cut tubers; 4 cc. of ethylene chlorhydrin in 1 liter of water; soaked 2 hours. McCormick. Lot *W*: Check, not treated. Lot *X*: Check, soaked 2 hours in water. Lot *Y*: Check, soaked 1 hour in water.

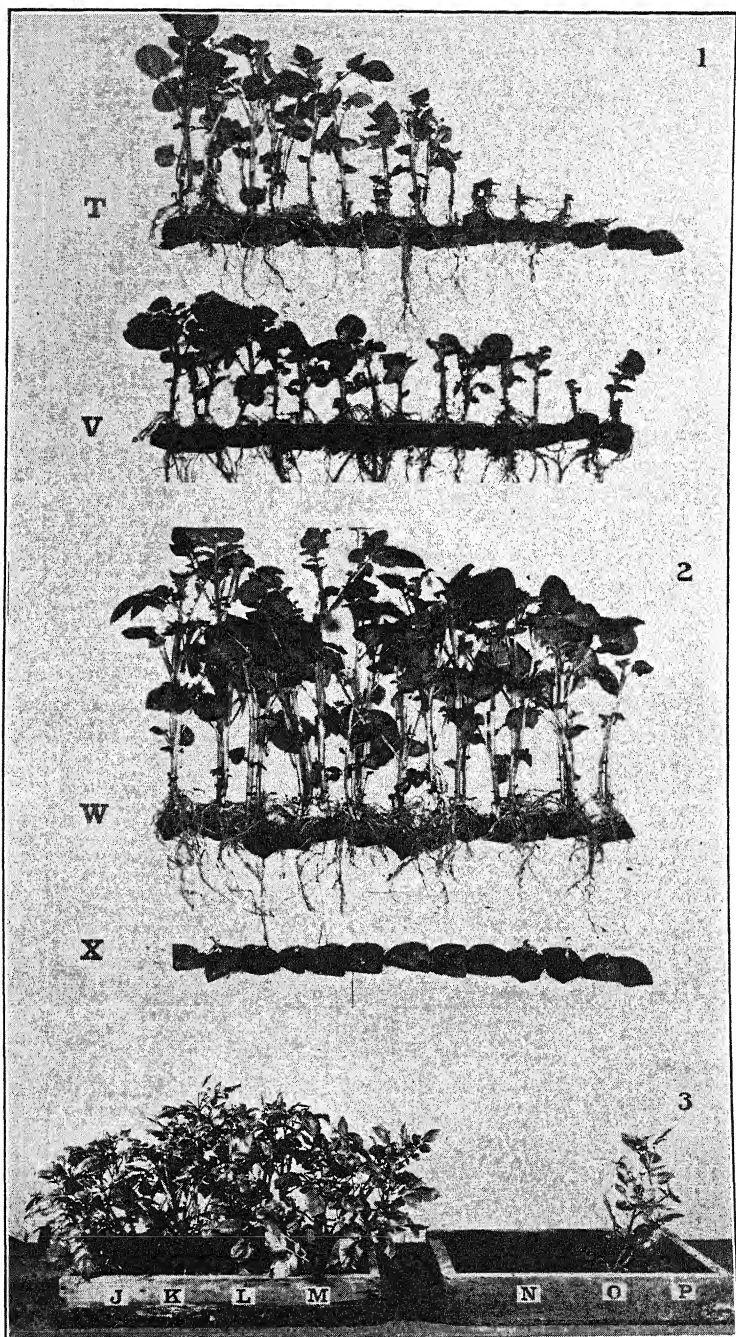
## PLATE XXXIV

FIG. 10. Lot *H*: Ethylene chlorhydrin, vapor method in special "gassing-house," using electric fan for stirring the air; 1,500 cc. of ethylene chlorhydrin in 4,250 liters of air space; treatment for 20 hours; whole tubers treated, allowed to stand in air one day after treatment, then cut and planted. McCormick. Lot *K*: Check, not treated.

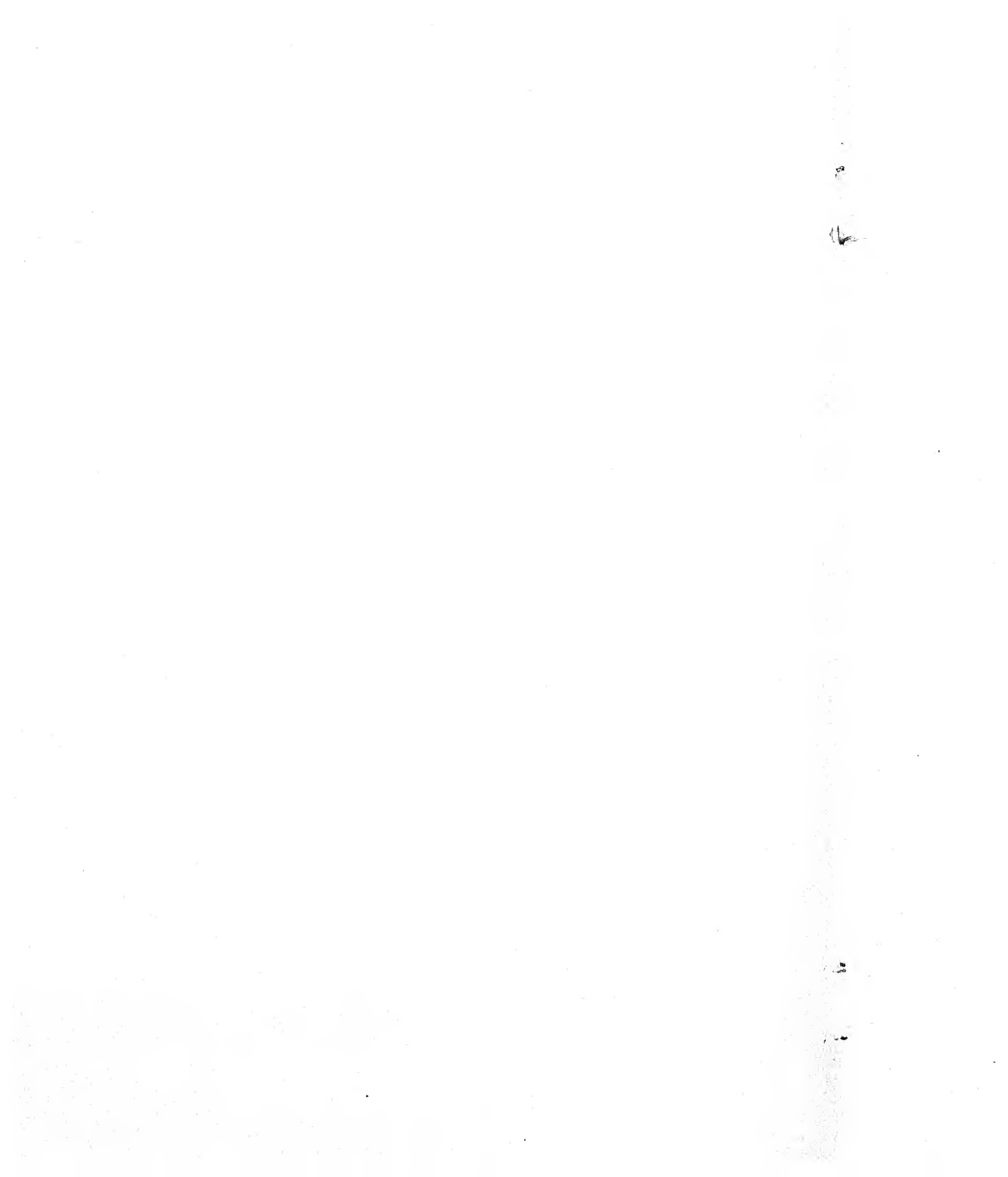
FIG. 11. Lot *C*: Ethylene chlorhydrin, vapor method, whole tubers treated; 1 cc. of ethylene chlorhydrin per liter of air space for 48 hours; tubers allowed to stand in air 7 days, then cut and planted. Bliss Triumph. Lot *Z*: Checks, allowed to stand in air 7 days, then cut and planted.

FIG. 12. Lot *S*: Ethylene chlorhydrin, vapor method, whole tubers treated; 3 cc. of ethylene chlorhydrin per liter of air space for 24 hours; tubers allowed to stand in air 21 days after treatment, then cut and planted. Bliss Triumph. Lot *W*: Checks, allowed to stand in air for 21 days, then cut and planted.

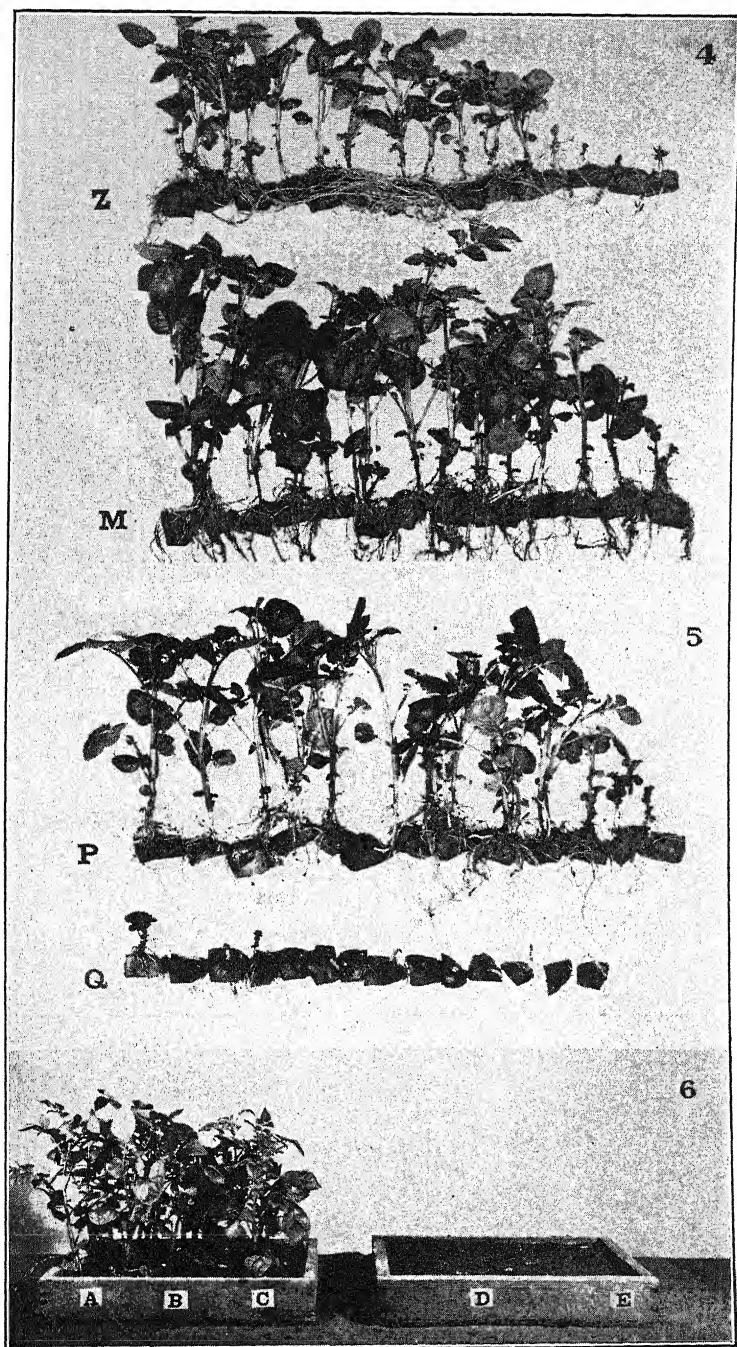




DENNY: SPROUTING OF DORMANT TUBERS



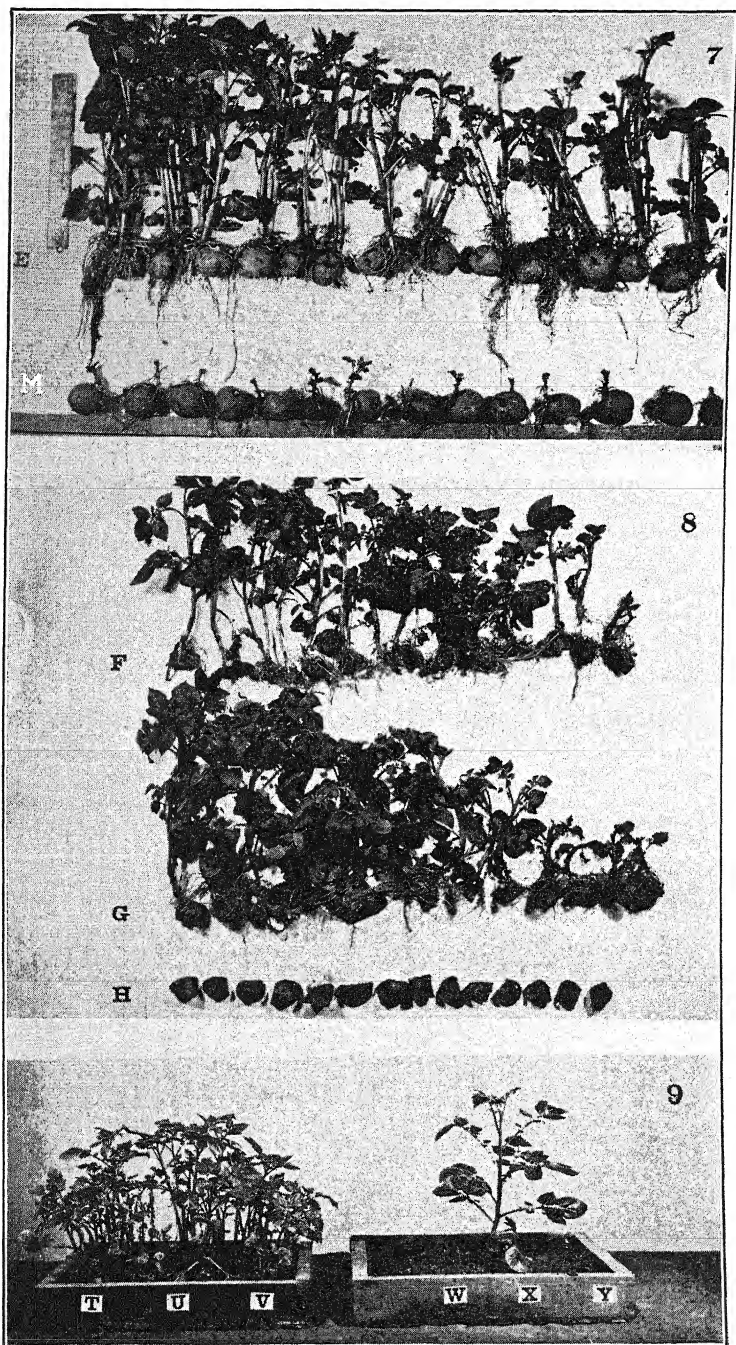




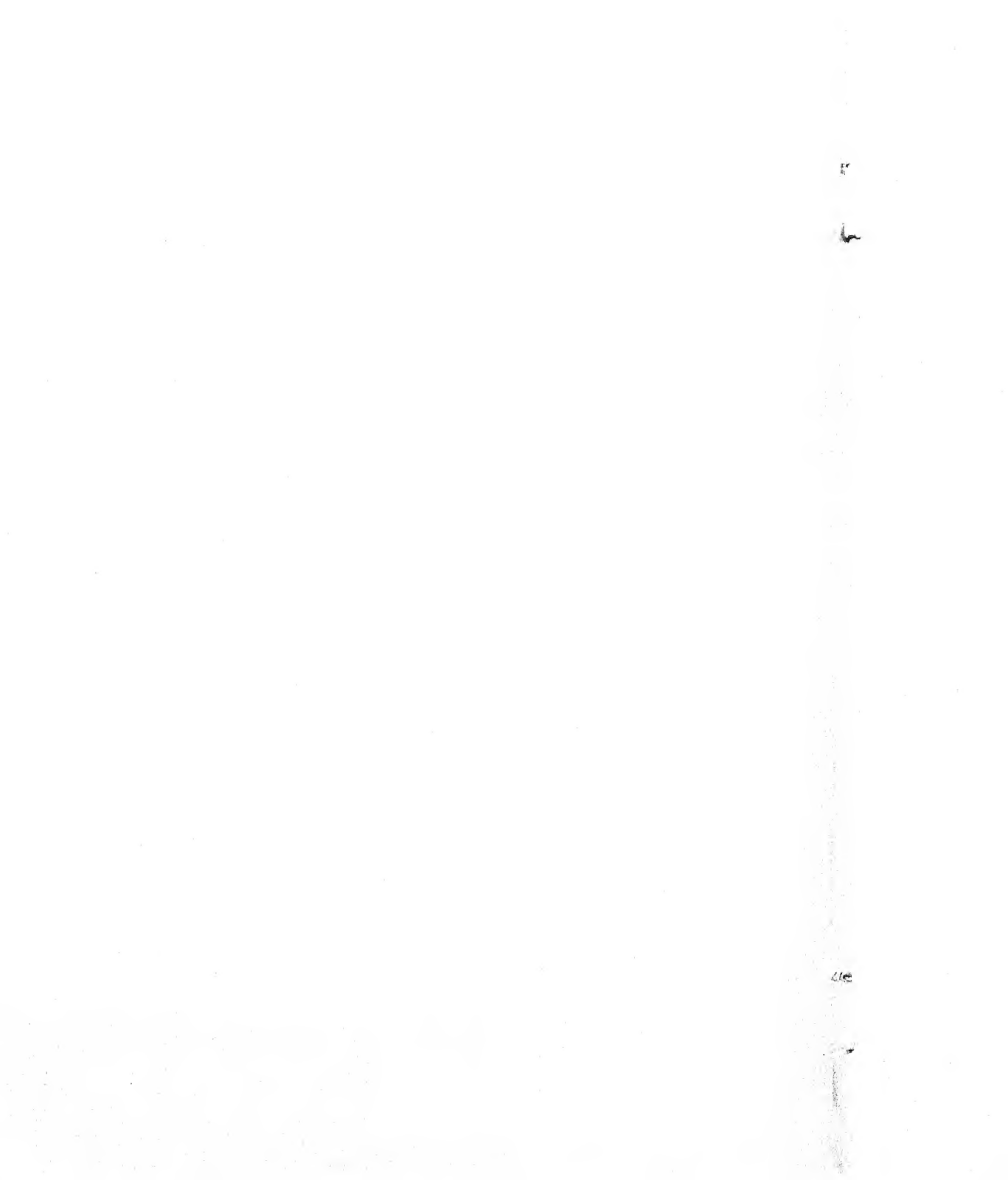
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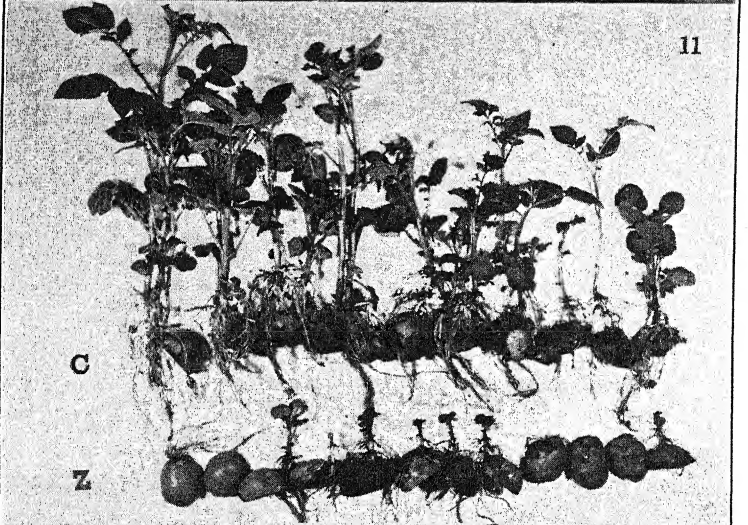


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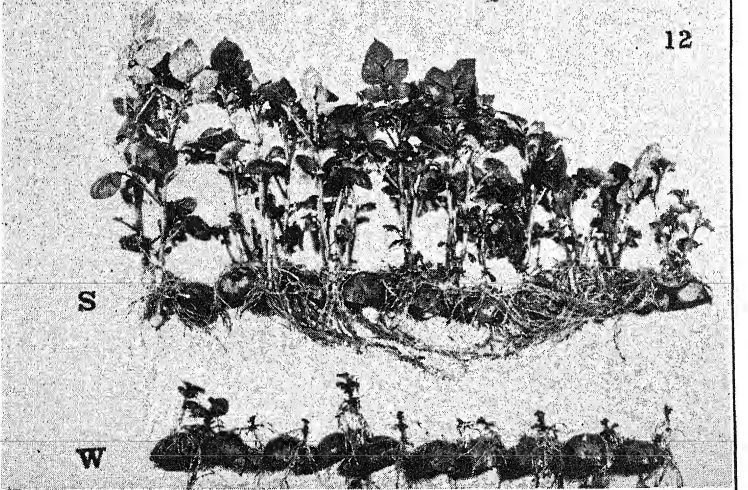




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DENNY: SPROUTING OF DORMANT TUBERS



## STUDIES ON ASTER YELLOWS<sup>1</sup>

L. O. KUNKEL

Contribution from Boyce Thompson Institute for Plant Research.

An infectious chlorosis known as "aster yellows," because it attacks the China aster, *Callistephus chinensis* Nees, is prevalent throughout the United States. The aster is grown extensively in other parts of the world, especially in Europe and the Orient, but the disease is known to be prevalent only in North America. Although it has been present here for many years and is a serious hindrance to aster growing, it has been given little attention by plant pathologists. This is partly, no doubt, because it belongs to that group of obscure plant maladies known as virus diseases. Another probable reason why pathologists have neglected aster yellows is that it is not known to attack any plant of great economic importance. Smith (26) gave a good description of the disease in 1902 and suggested that it might go to other plants in the Compositae closely related to the China aster. He was unable to find any parasitic organism associated with yellows and suspected its relationship to the virus disease group. Whether the incidence of aster yellows has changed during the past twenty-five years is not known. Smith speaks of it as "widespread and destructive." At the present time it is so serious in many sections of the country that the planting of asters is being greatly restricted or even abandoned. Aster plots showing 90 to 95 percent of yellowed plants are not uncommon throughout the eastern United States. One hundred percent infection has occasionally been observed in fields containing several hundred plants. In certain localities, however, the disease is not yet serious, and small plantings sometimes remain disease-free to the end of the season.

At the suggestion of Doctor William Crocker, the writer undertook a study of aster yellows in the spring of 1923. The work, started at that time, has been continued during the past three years. Special attention was given to the means by which the disease is transmitted to healthy plants, to its relationship to similar well known diseases of other plants, to its overwintering in wild host plants, to the life and habits of its insect carrier, to its incubation period in both plant and insect host, to its host range, to possible methods for its control, and to its etiology. Several brief reports covering certain phases of the work have already been published (12, 14, 15). The object of the present paper is to record in more detail observa-

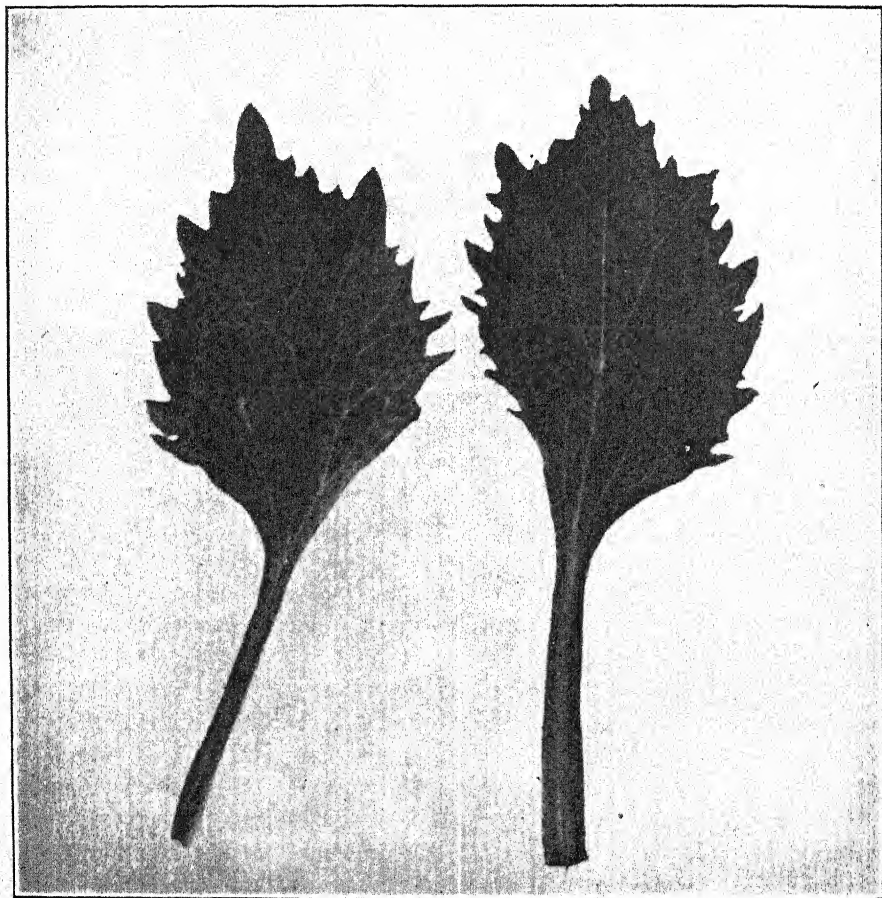
<sup>1</sup> Published, at the expense of the Institute, out of the order determined by the date of receipt of the manuscript.

tions and experimental results obtained to date and to discuss the relation of these data to the yellows-disease problem.

#### SYMPTOMS ON ASTER

Smith (26) has carefully described and pictured the yellows disease as he observed it on aster. A detailed description need not, therefore, be given here. A brief account of the development of the disease will, however, give opportunity to call attention to some of the more important symptoms.

Aster leaves affected with yellows never show mottling. The disease is, therefore, easy to distinguish from chloroses of the mosaic type. In advanced stages yellows is always systemic in the above-ground portions of the



TEXT FIG. 1. Showing "clearing of the veins" in affected leaves.

plant. The first symptom to be observed on a young plant is a slight yellowing along the veins in the whole or in a part of a single young leaf. This symptom, which will be referred to as "clearing of the veins" (text

fig. 1), has not been observed in connection with any other aster disease and makes diagnosis possible long before conspicuous chlorosis appears. As more leaves develop shortly after infection, they show the same clearing of veins. After a plant has been diseased for some time the new leaves are chlorotic throughout. The larger veins seem to be a barrier to the spread of yellows in leaves. The tissues on one side of a midrib or of any large leaf vein are frequently chlorotic while those on the other side are of a normal green color. One half or more, or a sector amounting to less than one half of a plant, may be chlorotic for some time before the remaining portion is affected. This shows that rapid spread around the stem of the plant does not take place in many cases. It is probable that such sectorial infections occur when the disease enters the stem some distance back of the apical bud and when longitudinal growth of the stem is approximately equal to the rate of spread of the disease. This is also indicated by the fact that lateral shoots sometimes show the disease before it appears in the terminal portions of the stem. While chlorosis does not appear in leaves that are mature when the plant is attacked, leaves that are from one half to two thirds full grown and are of a normal green color do become diseased, as is shown by clearing of veins and general chlorosis. This fact shows that the disease is capable of destroying chlorophyll. It is probable that it also depresses the production of chlorophyll in leaves developed from diseased buds. When the attack is severe the young leaves are almost white. Such leaves may become more or less green as they grow old. The disease causes chlorosis in all green portions of the plant. Strangely enough, however, petals which normally contain no chlorophyll become quite green when diseased. While yellows depresses chlorophyll-production in portions of the plant that are normally green, it causes the production of some green-colored substance in floral parts where chlorophyll is not normally present. Since this green substance is present in the cell sap, it is probably not chlorophyll. In different plants and under different conditions the disease causes widely different degrees of fading or yellowing of leaves varying from slight to extreme chlorosis. Some aster plants show much less chlorosis and are less stunted than others. They seem to resist the disease to a certain extent.

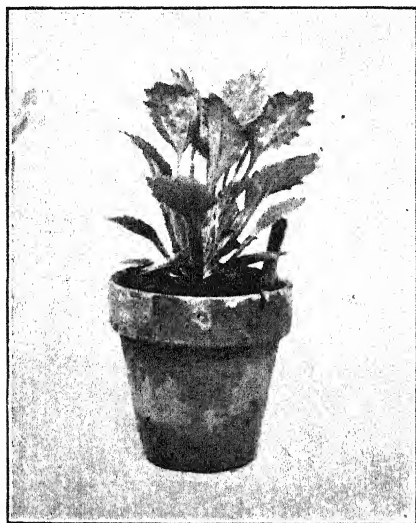
One of the most striking symptoms of aster yellows results from the abnormal production of secondary shoots. Such shoots frequently arise in the axils of leaves that show a normal green color and were mature before the plant became infected. They are always thin and have the appearance of etiolated branches.

Plants that contract the disease before they are mature are always more or less stunted. The degree of stunting varies with the age of the plant at the time it becomes infected and with the size of the sector infected. The effect of the disease on the main stem is greatly to shorten the internodes. It usually has the opposite effect on the secondary shoots. Diseased flowers and seeds are often much larger than healthy ones, but cases also



occur in which they are dwarfed. The flower heads are always more or less dwarfed. Individual flowers in some cases develop into vegetative branches, which may or may not bear small flower heads. Trichomes on diseased flowers frequently develop into leaf-like structures. The petals of ray flowers are uniformly much dwarfed. The root systems of diseased plants appear normal but are smaller than those of healthy plants.

Another interesting result of yellows is the change which it brings about in the response of the plant to gravity. Instead of diseased leaves lying flat and making a broad angle with the vertical, they stand upright, as is shown in text figure 2. A similar response is shown by individual flowers



TEXT FIG. 2. Upright position of leaves in affected plants.

and branches of diseased plants. This modification of the response of plants to gravity sometimes occurs in the case of certain plants infected with rust and smut diseases where mycelia are systemic.

Yellows also causes certain morphological changes. Diseased leaves are frequently somewhat deformed. Their petioles are longer than those of healthy leaves of the same age. The leaf blade on the other hand is narrower, and on the whole smaller, than the normal blade. Diseased leaves may have deep clefts and notched margins but are seldom severely deformed.

Necrosis is a symptom of advanced stages of yellows. It is most severe in the stem tissues a short distance below the apical bud of the main stem or of a branch. It causes the collapse of certain tissues but does not kill the plant. Diseased plants that escape infection by fungi will live as long, or almost as long, as healthy ones.

In summarizing, it may be said that the yellows of asters is an infectious disease causing diffuse and well marked chlorosis, clearing of veins, occasional

one-sided or sectorial infection, upright habit of growth, malformation and increased growth in certain organs, dwarfing of the plant as a whole, and the abnormal production of secondary shoots.

#### INSECT TRANSMISSION

Because of the similarity between aster yellows and certain insect-borne virus diseases, such as curly-top of beet, sugar cane and corn mosaic, the writer was led to suspect that its spread might be due to insects. Observations made on aster plots at the Boyce Thompson Institute at Yonkers, New York, during the summer of 1923 showed that at the time the disease was spreading rapidly the tarnished plant bug, *Lygus pratensis* L., and two leafhoppers, *Cicadula sexnotata* Fall., and *Empoasca flavescens* Fab., were present in large numbers. Two other leafhoppers, *Agallia sanguinolenta* Prov. and *Graphocephala coccinea* Forst., were present but not abundant. Aphids were found in small numbers but were never numerous. The same insects were present in other aster fields in the vicinity of New York City. Because of their activity and abundance, leafhoppers and the tarnished plant bug were suspected of spreading the disease.

In order to test the insect hypothesis of transmission, a number of experiments were undertaken. The aster variety, Late Branching, obtained from Vicks' Sons of Rochester, New York, was used in all experiments here reported.

#### Experiments with the Tarnished Plant Bug, *Lygus pratensis* L.

Ten adult tarnished plant bugs were transferred from diseased aster plants to each of 3 insect-proof cages. Each cage contained 4 healthy and 2 yellowed aster plants. Four healthy and 2 yellowed aster plants in another insect-proof cage served as checks. The bugs lived and reproduced in each of the 3 cages to which they were transferred, but the 12 healthy plants in these cages as well as the 4 healthy check plants remained healthy for 3 months, when the experiment was ended.

Ten adult tarnished plant bugs were transferred from diseased aster plants to each of 4 insect-proof cages. Each cage contained 6 healthy young aster plants. Twelve other plants of the same age growing in 2 other cages served as checks. The bugs lived and reproduced in each of the 4 cages to which they were transferred, but all plants remained healthy during the 3 months that they were kept under observation.

After being compelled to feed for one week on yellowed aster plants in insect-proof cages, 25 tarnished plant bugs were transferred to each of 3 insect-proof cages containing 2 yellowed and 3 healthy young aster plants. The bugs lived and reproduced. They caused considerable injury to the plants through their punctures, but all the healthy plants remained healthy during the 3 months that they were kept under observation.

Ten tarnished plant bugs reared on yellowed aster plants in insect-proof

cages were transferred to each of 7 insect-proof cages containing 6 healthy young aster plants each. The bugs lived and reproduced in each of the cages, but all plants remained healthy during a period of a little more than 3 months that they were kept under observation.

Five tarnished plant bugs reared on yellowed aster plants were transferred to each of 3 insect-proof cages containing 6 healthy young aster plants each. The bugs lived and were observed to feed, but all plants remained healthy during the 3 months they were kept under observation.

These negative results with the tarnished plant bug together with confirmatory field evidence indicate that this insect is unable to transmit aster yellows.

#### Experiments with the Green Aster Leafhopper, *Empoasca flavescens* Fab.

A small green leafhopper closely resembling the potato leafhopper, *Empoasca fabae* (Harr.), was abundant on asters in the vicinity of New York City during the past three seasons. It was submitted to several authorities on this group of insects for identification. They did not agree in their opinions regarding the species to which it belongs. Prof. Herbert Osborn, to whom specimens were sent, reported that it is probably *Empoasca flavescens* Fab. His opinion is in agreement with that of Dr. Albert Hartzell, who carefully compared it with other species of *Empoasca*. This identification has, therefore, been accepted.

Although the green aster leafhopper closely resembles the potato leafhopper, it seems to be distinct from this species for the following reasons. Leafhopper cultures taken from asters and from potatoes grown in close proximity in the same field prove that the green aster leafhopper does not produce colonies on the potato and that the potato leafhopper does not live and breed on the aster. The aster leafhopper, when forced to feed on potato plants, produces a whitish stippling on the leaves just as it does on aster leaves. The potato leafhopper does not produce such stippling either on potato or on aster. Moreover, the potato leafhopper when grown on potato plants causes the disease known as hopperburn, while the aster leafhopper when cultured in the same way causes no hopperburn.

The green aster leafhopper was first cultured on asters in insect-proof cages in July, 1923. Twenty-five adults were transferred from plants growing in field plots where yellows was abundant to each of 5 insect-proof cages. Each cage contained 6 healthy young aster plants. Six healthy young plants of the same age kept in a similar cage served as checks. The leafhoppers thrived and reproduced in each of the 5 cages, but the 30 plants on which they fed as well as the 6 check plants remained healthy during the 3 months the experiment was in progress.

The green aster leafhopper was kept in culture on aster plants in insect-proof cages for a little more than 2 years. It reproduced continuously during this period, passing through many generations. The colonies

flourished in the winter as well as in the summer. Insects reared on yellowed aster plants were transferred at different times to healthy young plants grown in cages. Several hundred plants were exposed in this way during the 2 years this leafhopper was cultured. From 25 to 300 adults reared on yellowed plants were kept for varying periods of time on healthy young aster plants. The details of these experiments will not be given, as all results were negative. The green aster leafhopper seems unable to transmit the disease. Similar but less extensive experiments were made in attempts to transmit yellows to aster by means of the potato leafhopper, *Empoasca fabae*. This leafhopper also failed to transmit the disease.

#### Experiments with the Clover Leafhopper, *Agallia sanguinolenta* Prov.

In March, 1924, many blocks of frozen soil containing rye plants from seed sown the previous autumn between rows of aster plants were brought into a warm laboratory. The object of this experiment was to determine, if possible, whether any of the aster leafhoppers live over winter in the adult stage. It was hoped that if adults were present in the soil around the rye plants they would become active when brought into the laboratory. Only one species of leafhopper, *Agallia sanguinolenta*, was obtained in this way. The blocks of soil were put into flats and each placed near a window. The plants and the windows were examined twice daily for the presence of leafhoppers. After from 1 to 3 days, adults of the clover leafhopper were found on the soil in the flats or on the windows above the flats. No other leafhopper was obtained. Many blocks of soil failed to yield any leafhoppers, but a total of 13 adult clover leafhoppers were secured. It is interesting to note that all these adults were of exactly the same size and somewhat smaller than adults of the species present during the summer. This observation suggests that only those individuals that moult for the last time just before cold weather sets in, in the fall, are able to live through the winter. The 13 clover leafhoppers obtained in this way were placed on healthy young aster plants. They reproduced, and from them a strong colony was obtained. It was kept in culture on asters for a little more than one year. During this time it passed through many generations. Suitable colonies reared on yellowed aster plants were repeatedly transferred to healthy aster plants, but in no case did they transmit the yellows disease. Although the clover leafhopper flourishes on aster, it was finally concluded that it takes no part in the spread of yellows.

#### Experiments with the Peach Aphid, *Myzus persicae* Sulz.

This aphid is seldom found in large numbers on field-grown aster plants, but the *Calendula*, which is very susceptible to aster yellows, is one of its favorite hosts. If infested *Calendula* plants are caged with aster plants, the aphids will spread to and multiply on the asters.

Fifty peach aphids, consisting of both adults and nymphs taken from

yellowed *Calendula* plants, were placed on 6 healthy young *Calendula* plants in an insect-proof cage. At the same time, 25 similar aphids were placed on each of 6 other healthy young *Calendula* plants in another cage. Six other healthy *Calendula* plants of the same age kept free of insects served as checks. All plants remained healthy during the 2 months that they were kept under observation.

Approximately one half cubic centimeter of peach aphids, consisting of both nymphs and adults taken from a yellowed aster plant which had been infested for a period of 2 weeks, were placed on a healthy young aster plant. After a period of 2 weeks, all aphids were killed by fumigation. The plant remained healthy for 2 months, when the experiment was ended.

Four cubic centimeters of peach aphids taken from yellowed aster plants were placed on 12 healthy young aster plants in an insect-proof cage. At the same time the same quantity of peach aphids from the same source was placed on 4 other healthy young aster plants in a second cage. The aphids were allowed to feed on the exposed plants during 6 weeks that the experiment was in progress. All plants remained healthy.

Approximately one half cubic centimeter of peach aphids reared on yellowed *Calendula* plants were transferred to each of 12 healthy young *Calendula* plants, and to one healthy young aster plant. The plants were kept in separate lantern-globe cages. Twelve healthy *Calendula* plants of the same age kept in a large cage served as checks. Five days after the experiment was started all insects were killed by fumigation. All the plants remained healthy for a period of 2 months, when the experiment was ended.

The above-described experiments bring evidence that the peach aphid is unable to transfer yellows to either of the two very susceptible host plants, aster and *Calendula*.

### Experiments with Other Insects and with Mites

Yellowed aster plants infected with the greenhouse thrip, *Thrips tabaci* Lind. with the white fly, *Trialeurodes vaporariorum* W., and with the red mite, *Tetranychus telarius* L., were on several different occasions placed in cages containing healthy young aster plants. These insects and the mites always spread to the healthy plants, but in no case was yellows transmitted.

### Experiment with the Leafhopper, *Graphocephala coccinea* Forst.

The beautiful large leafhopper, *Graphocephala coccinea*, occurs in small numbers on the aster. During the summer of 1923, 10 adults caught from yellowed plants in aster plots where yellows was abundant were transferred to a cage containing 6 healthy young aster plants. Six of these insects were still alive after 2 months, but they did not reproduce. All plants remained healthy during the 2 months they were kept under observation. Since this leafhopper was present in small numbers and did not reproduce on asters, it was not used in later experiments. While the experiment does not prove

that this *Graphocephala* can not transmit yellows, it at least shows that the insect does not readily transmit the disease.

### Experiments with the Leafhopper, *Cicadula sexnotata* Fall.

Adults of this species were first observed by the writer on asters late in May, 1923. By the end of July they were present in considerable numbers. As it was evident that the aster is one of its favorite host plants, experiments were undertaken to test its relation, if any, to the spread of aster yellows.

On July 31, 25 adults taken from an aster plot containing many yellowed plants were placed in each of 2 insect-proof cages containing 6 healthy young aster plants each. One week later, 50 adults taken from the same aster plot were placed in each cage. The cages were numbered 1 and 2. The insects lived and reproduced in both cages. A third cage containing 6 similar healthy young plants served as a check. Eighteen days after the experiment was started, 3 of the plants in cage 2 showed yellows. Eight days later, a fourth plant became diseased. By September 10, all 6 plants were yellowed. On this date 2 healthy young plants were placed in the cage. On October 1, one of these plants showed the disease. Six days later the other plant became yellowed. Thirty-one days after the experiment was started one plant in cage 1 showed the first symptoms of yellows. The other 5 plants and the 6 check plants remained healthy during the two and one half months the experiment was in progress. The experiment shows that *Cicadula sexnotata* transmits the aster yellows disease.

In a second experiment, started August 9, 10 adults of this species were placed in cage 4 containing 6 healthy young aster plants. Twenty-five adults were placed in cage 5, which also contained 6 healthy young aster plants. The insects were caught in an aster plot containing many yellowed plants. Six healthy young aster plants in another cage served as checks. The leafhoppers flourished and reproduced in both cages. Eighteen days after the experiment was started one of the plants in cage 5 showed yellows. Three weeks later a second plant became diseased, and by September 3 a third plant was diseased. When the disease first appeared in this cage, 20 healthy young seedling aster plants were placed in the cage. Four weeks later 7 of the 20 seedlings showed yellows. Three of the large plants and 13 of the small seedlings remained healthy to the time the experiment was ended. The 6 plants in cage 4 and the 6 check plants remained healthy during the two and one half months the experiment was in progress. The insects in cage 5 transmitted the disease while those in cage 4 did not.

The leafhoppers lived and reproduced in the 4 cages in which they were placed. One hundred adults taken from cage 2, in which all plants had become diseased, were placed in cage 7 containing 31 healthy young plants. One hundred adults taken from cage 4, in which all plants had remained healthy, were transferred to cage 8 containing 32 other healthy young plants. The insects thrived in both cages. The experiment was started October 11.

By November 20, 40 days after the experiment was started, 19 of the 31 plants in cage 7 had yellows. One week later all the 31 plants showed the disease. The 32 plants in cage 8 remained healthy. The experiment shows that the colony of insects from cage 4 does not transmit yellows while that from cage 2 readily transmits it to healthy plants.

From December, 1923, to March, 1924, several transmission experiments in which *Cicadula* was used were carried out under carefully controlled conditions. In these experiments 183 aster plants were exposed to leafhoppers which had previously fed on yellowed aster plants. Of these plants 142 became diseased while 41 remained healthy. Fifty-eight check plants exposed to leafhoppers that had not fed on diseased asters did not take the disease.

The above-described experiments prove that *C. sexnotata* readily transmits aster yellows from diseased to healthy aster plants. They also show that colonies that have never fed on yellowed plants are unable to cause the disease.

#### *Cicadula sexnotata* Fall.

The small gray leafhopper found in abundance in aster fields in the vicinity of New York City was identified by several authorities to which it was submitted as *Cicadula sexnotata* Fall. It is present in North America from Alaska to the southernmost borders of the United States. It is also widespread and common throughout Europe. It occurs in Japan and probably throughout the Orient. In what part of the world it is endemic is not known. There is some evidence that the species has been introduced into North America. It was first reported in the United States by Forbes in 1884. Professor Herbert Osborn (21) found a specimen in the Harris collection of the Boston Society of Natural History which he thinks was collected between 1840 and 1850. Because several collectors failed to report its presence prior to the early eighties, Professor Osborn suggests that it may be an introduced species. He thinks it unlikely that this leafhopper could have been missed by such early collectors as Say, Fitch, and Uhler, if it had been so widespread and abundant as it is at the present time. It seems probable that it was introduced into this country less than one hundred years ago. It has been a common insect here for almost fifty years. Since aster yellows is not known in Europe, it was thought advisable to determine whether the *C. sexnotata* of Europe is identical with that prevalent in the United States. Specimens from cultures used in the experiments here reported were sent to Dr. A. D. Cotton of the Royal Botanic Gardens at Kew, England, with the request that they be compared with English specimens of the species. Dr. Cotton sent the specimens to the Keeper of Entomology at the British Natural History Museum at London. They were compared with English specimens by Mr. China, who reports that he can find no specific distinction between the British and the American specimens. A slight difference in color markings was observed, but, con-

sidering the variability in this respect of the British forms, Mr. China states as his opinion that the slight difference indicates at most only a slight variation. The writer is indebted to Dr. Cotton, Mr. China, and the Keeper of Entomology of the British Natural History Museum for this comparison and statement.

One of the reasons why this species of *Cicadula* is so abundant is that it feeds and breeds on a wide range of host plants. Osborn (21) reports it from grasslands and oat fields of Maine and mentions it as a serious pest on wheat, oats, and barley crops in the northwest (19). It is said to have caused serious damage to wheat in the province of Östergötland, Sweden, in the summer of 1918 (7). From Bohemia it is reported as injurious to sugar beets (31). It is well known throughout Germany as the cause of serious injury to grasses, cereals, and certain leguminous plants (10).

Osborn has given a careful description of its life history together with drawings of its eggs, of the five instars of nymphs, and of the adult (20, 21). Photographs of an adult and of nymphs in the first, second, and fifth instars are shown in figures A, B, C, and D, Plate XL. The only point in the life history of this insect which is not clear is its manner of passing the winter. This is a matter of considerable importance in connection with the overwintering of the aster yellows disease.

During the past three years the writer has kept this leafhopper in culture continuously in insect-proof cages. Twenty-five adults caught July 31, 1923, furnished the beginning of these cultures. No serious difficulty has been experienced in maintaining the cultures in a vigorous condition during all seasons of the year, except on one occasion when the temperature of the greenhouse in which the cages were kept went too low for a short period of time. The chief factor to be given attention is that a proper balance be maintained between number of insects and number and size of host plants. The cultures must also be kept free of insect enemies and fungous parasites. During the 3 years that *Cicadula* has been cultured it has passed through at least 25 generations. The females deposit eggs over a long period of time. Those of one generation are still depositing eggs when the females of the next generation start laying. No evidence has been found of distinct broods either in the field or in cultures. In a greenhouse kept at a temperature of 70° to 75° F. it will pass through its life cycle from egg to egg in about 40 days. The maximum or even the average number of eggs laid per female is not known. One female kept under careful observation during her life of 87 days laid eggs from which were hatched 127 nymphs. The average age reached by insects kept under favorable conditions at a temperature of 70° to 75° F., when only a few insects are kept in each culture and suitable food plants are supplied at least once a week, is about 120 days. When large numbers are kept in each cage the average length of life is not more than 60 days, even when great care is taken to provide suitable food plants at frequent intervals.



There is considerable variation in the length of time necessary for the eggs to hatch. In a culture kept in constant subdued light at a constant temperature of 68° F. and a relative humidity of 80 percent of saturation, the minimum period required for eggs to hatch proved to be 10 days. Under ordinary greenhouse conditions at temperatures ranging from 70° to 75° F., some eggs hatch in from 10 to 11 days after deposition while others require varying periods of time up to 3 weeks.

The eggs are deposited under both the upper and the lower epidermis of aster leaves. Some are deposited in the leaf petioles and even in branches and main stems. Most of them, however, are placed between the lower epidermis and the mesophyll of leaves. The eggs usually lie with their long axes in a plane parallel to the leaf surface, but occasionally they are placed obliquely to the leaf surface and lie with the distal end deep in the mesophyll. A waxy substance of a gray color plugs the hole made in the epidermis of the leaf when the egg is deposited. The eggs are frequently found in groups of three or four, but more often they occur singly. Plant cells adjoining the eggs remain turgid and normal in appearance from the time the eggs are deposited until they hatch. Eggs deposited in plants kept constantly in the dark failed to hatch. Whether this failure is due to the direct effect of darkness on the eggs or indirectly to some effect on the plant tissues in which they are deposited is not known.

In cultures kept at about 70° F. most of the eggs hatch in from 11 to 13 days. Most of the nymphs are in the second instar by the 16th day. By the 27th day some are in the fifth instar but many are still in the third and fourth instars. The first adults usually appear on the 31st day. Many are adult by the 33d day, but at least 10 more days must elapse before all insects reach the adult stage.

Both nymphs and adults vary considerably in color in cultures and in the field. Some are almost black while others are a light greenish-gray color. Ten dark-colored adults were collected and placed together in a culture. Their progeny contained no more dark-colored individuals than the progeny from light-colored adults.

*Cicadula sexnotata* will live and reproduce on a very large number of different host plants. Some of its favorite hosts are: aster, lettuce, sow thistle, great ragweed (*Ambrosia trifida* L.), daisy fleabane (*Erigeron annuus* (L.) Pers.) and other *Erigerons*, English plantain (*Plantago lanceolata* L.), dandelion (*Taraxacum officinale* Weber), wheat, oats, rye, barley, *Calendula*, *Ammobium alatum*, *Matricaria alba*, *Centaurea imperialis*, *Gaillardia grandiflora*, Moon Penny daisy (*Chrysanthemum leucanthemum*), and the African daisy (*Dimorphotheca aurantiaca*). Some plants on which they do not flourish are tobacco, potato, tomato, *Schizanthus*, peach, *Begonia*, and alsike clover. There are many plants on which they can live and reproduce, but on which they will not congregate when more favored host plants are available. Some examples of these are corn, African marigold, milkweed (*Asclepias nivea*), and cosmos.

An effort was made to determine how *Cicadula sexnotata* passes the winter. The method, already described, by which the clover leafhopper was shown to live over winter and was obtained from blocks of frozen soil in March, 1923, failed to yield a single specimen of *Cicadula*. Since it was present in large numbers in the field from which the blocks were taken, it should have been obtained by this method if it had been able to live through the winter. Both adults and nymphs, when subjected to temperatures of 5° C. or lower, die in a few hours. All evidence obtained indicates that they are unable to live through the winter.

On March 13, 1926, while the weather was quite cold, small blocks of soil containing a number of rye plants grown from seed sown the previous autumn were transferred to each of 8 different lantern-globe cages. Other blocks of soil with similar rye plants were placed in each of 2 flats and put into a large insect-proof cage. All cages were kept in a greenhouse. The lantern globes were not removed and the door of the large cage was never opened until the plants had been caged for 25 days. Each culture was then carefully examined. Eleven nymphs of *C. sexnotata* were found on the plants in the large cage. The 8 lantern-globe cultures were carefully examined and gave the following results: Culture number 1 contained two adults; culture number 2 was insect-free; culture number 3 contained one nymph in the fourth instar; culture number 4 contained one nymph in the second and one in the third instar; culture number 5 contained two nymphs in the fourth instar; culture number 6 contained one nymph in the fourth and one in the fifth instar; culture number 7 was insect-free; culture number 8 contained one nymph in the first instar.

No leafhoppers were obtained from similar rye plants held in a warm room for 4 days. This indicated that neither nymphs nor adults were present in the soil or on the plants when taken from the field. The insects that were present after 25 days could not have come from any source except the soil or the rye plants. While eggs were not actually observed in the rye plants, the experiment brings indirect proof that the species passes the winter in the egg stage. All nymphs obtained in the cultures used in the above experiment were kept to maturity and identified as belonging to *Cicadula sexnotata*.

The feeding habits of *Cicadula* were carefully studied. Insects caught in the field are wild and difficult to observe closely, but insects reared in lantern-globe cultures and transferred frequently become so tame that their bodies can be lifted by means of a needle without causing them to stop feeding. They try to feed on any portion of a leaf on which they alight. If, however, they fail to find a leaf vein they soon move to another place. After a suitable vein has been found they will frequently continue to feed in one place for more than an hour. As they pierce the tissues the posterior end of the body is sometimes moved sidewise through an angle of as much as 180°. This movement apparently aids the insect in boring into the tissues.

If large numbers of insects are placed on an aster plant, their feeding will cause wilting, discoloration, and other symptoms of injury. The severity of this effect depends on the size and age of the plant and on the number of insects. Plants soon recover from such direct effects if the insects are promptly removed. That this type of injury has no relation to the aster yellows disease is shown by the fact that virus-free as well as virus-bearing insects are equally effective in producing it, while only virus-carrying insects are capable of causing the yellows disease. Moreover, direct injury appears in a few hours after plants are exposed and is proportional to the number of insects and to the time during which they feed. Aster yellows on the other hand does not appear until after a definite incubation period averaging from 16 to 18 days, and its severity is in no way proportional to the number of insects used in transmitting it or to the time during which they feed.

#### MATERIALS, APPARATUS, AND METHODS

In the experiments described below, vigorous young aster plants of the variety Late Branching were used except where otherwise stated. Insects from vigorous colonies were employed in all tests.

The experiments carried out during the first year of work were conducted in large insect-proof cages. During the second and third years the large cages were used for stock cultures only. They were not used in transmission experiments. It was found impossible to prevent entirely the escape of insects when the doors of these cages were opened for the purpose of watering plants or of removing plants or insects. For this reason a lantern-globe cage such as is shown in text figure 3 was used in all transmission experiments conducted during the past two years. The lantern-globe cages were covered with cheesecloth or with copper-wire cloth having fifty meshes to the inch. They were sometimes set over plants grown in large crocks. More often, however, they were set on glass plates over 6-inch pots containing young plants. During the time of exposure the caged plants were kept in a greenhouse. Insect transfers were made only in the laboratories. Virus-free insects were transferred in one laboratory; virus-bearing insects in another. The lantern-globe cages containing insects were never opened except in the laboratories. They were always opened before windows having a northern exposure. As *Cicadula sexnotata* is strongly attracted by light, any insect that escaped when a cage was opened went to a window. It could then be caught and placed in a tube with other members of its colony.

A transfer tube consisting of a glass test tube 8 inches long and about one inch in diameter was used for confining insects temporarily during the transfers. The test tube was fitted with a cork or rubber stopper through which a small glass tube, open at both ends, was inserted. The small tube was about 3 inches long and one half inch in diameter. It was placed so as to extend approximately one inch below the small end of the stopper. Insects were conducted into the large test tube through the small open tube.

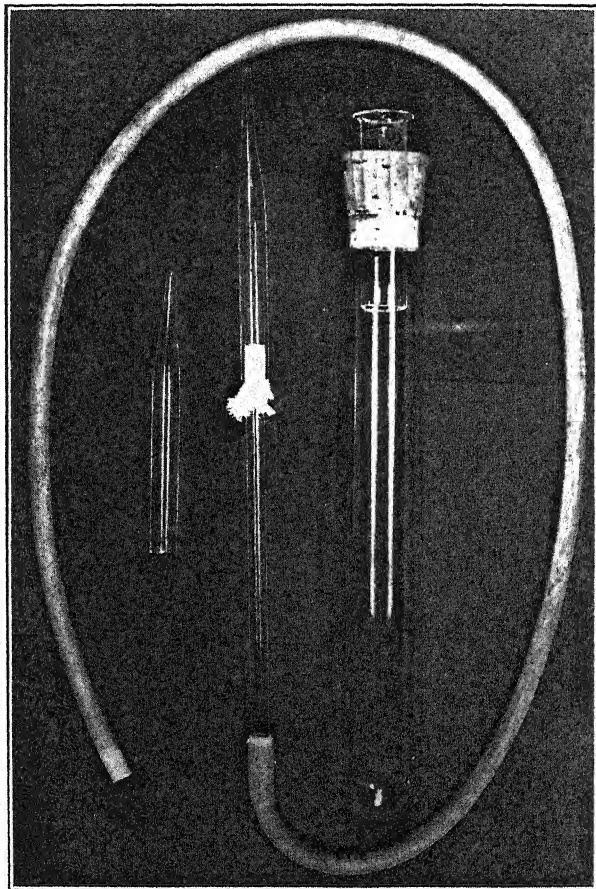
They do not readily escape from such a tube but may be removed when needed by means of an insect catcher. In case they were confined for a long period of time, a loose-fitting cotton plug was placed in the small tube.



TEXT FIG. 3. Showing method by which insects are confined to plants exposed to aster yellows.

All insect transfers were made by means of an insect catcher. This is a simple device made as follows. A piece of rubber tubing about 30 inches long and five sixteenths of an inch in outside diameter was fitted over one end of a small piece of glass tubing 5 inches long and one fourth of an inch in outside diameter. Two small pieces of cheesecloth were placed over the other end of the small glass tube, making a cap. One end of a second glass tube about 5 inches long and nine thirty-seconds of an inch inside diameter was fitted over the cloth-capped end of the smaller tube.

The other end of this tube was heated and drawn out, so as to have an inside diameter of approximately three thirty-seconds of an inch. It is important that the two glass tubes be of such diameter that the one over which the cheesecloth is placed fits easily yet snugly into the other. Both the transfer tube and the insect catcher are shown in text figure 4. When catching leafhoppers the free end of the rubber tube was placed in the



TEXT FIG. 4. Transfer tube and insect catcher.

mouth. The end of the glass tube having the small opening was placed near the insect to be caught, and air was quickly sucked through the tube. In this way insects are drawn through the small opening on to the cheesecloth. They are blown out by forcing air through the tube in the opposite direction. The catcher and transfer tube are convenient for catching and transferring, from cage to cage, leafhoppers, aphids, and other small insects. With this apparatus the writer was able to catch and transfer to a large

number of different lantern-globe cages as many as 2,800 leafhoppers in one day without the escape of a single insect.

In experiments on the transmission of yellows, plants were exposed to definite numbers of insects for definite periods of time. At the end of the period of exposure the insects were removed. Some insects were often lost by death during this time. The dead insects could usually be found, but in some cases, especially when the period was long, it was difficult to locate them. In order to be sure that no living insects remained on the plants after the termination of their exposure, they were fumigated with hydrocyanic acid gas immediately after being taken from the cages. Such fumigation readily kills leafhoppers. All exposed plants were fumigated at intervals of a few days during the time they were kept under observation in order to kill any insects hatched from eggs deposited during the exposure.

### Virus-free Colonies

It has been necessary for experimental purposes to maintain virus-free colonies of insects. These have been obtained in a number of different ways, as follows: (1) by rearing colonies from virus-free females captured early in the spring; (2) from insects hatched from dead rye and aster leaves; (3) from insects hatched from rye or wheat plants; (4) from insects hatched from aster plants that escaped infection; and (5) from insects hatched and taken from aster plants before yellows appeared.

On June 18, 1924, adults caught from lettuce growing in a garden were caged separately on 50 different healthy young aster plants. All the 50 plants remained healthy. Twenty-eight of the insects were females, and each produced a numerous progeny. All these colonies were maintained on aster plants for a period of 3 months. They all proved to be virus-free.

Before it was known that virus-free colonies could be reared from virus-carrying parents caged on wheat or rye plants, efforts were made to hatch eggs from dry leaves and leaf sheaths of rye plants and from dead aster leaves. The dry rye and aster leaves containing eggs were removed from the plants that bore them and placed on moist soil around healthy aster plants kept in cages. Many of the eggs in these dry leaves hatched. The nymphs went to the healthy aster plants and gave rise to colonies that always proved to be virus-free.

The third method of obtaining virus-free insects was the one most often used. It consists in caging virus-carrying insects on rye plants and taking their offspring to other cages before they become adult. In 39 different experiments, colonies of virus-free insects have been obtained in this way from virus-carrying parents. Virus-free colonies were started from each of the 5 different instars of the nymphs. Since this method of obtaining virus-free insects was learned, colonies have been maintained continuously on rye plants. Whenever virus-free insects were required, nymphs were taken from the colonies. The experiments show that wheat and rye, both of

which are favorite host plants for *Cicadula*, are immune to aster yellows. They also prove that the disease is not transmitted through the egg of the carrier insect or by contact of adults with nymphs.

Ten separately caged virus-carrying females were transferred daily for a period of 2 weeks. Several of the plants on which they fed, and in which they deposited eggs, escaped infection. Each plant was kept in a separate insect-proof cage. The colonies that developed on the plants that escaped infection proved to be virus-free.

Twenty colonies of virus-carrying insects were placed on 20 different healthy aster plants in separate insect-proof cages. After 2 days the insects were removed. After 14 days nymphs were present on all the plants. At this time 9 of the plants clearly showed early stages of infection, while the other 11 appeared healthy. The colonies on these 11 plants were removed and caged separately on 11 other young aster plants. Four of the colonies proved to be virus-free; the other 7 were contaminated.

No attempt has been made to obtain virus-free colonies by removing newly hatched nymphs from diseased plants, as was done by Stahl and Carsner (28) in their work with the sugar-beet leafhopper.

#### THE HOST RANGE OF ASTER YELLOWS

The aster yellows disease has been experimentally transmitted by means of *Cicadula sexnotata* to more than 50 different species in 23 different families of plants. It has been taken to one or more species in each of the following families: Compositae, Dipsaceae, Plantaginaceae, Martyniaceae, Gesneriaceae, Scrophulariaceae, Solanaceae, Labiatae, Boraginaceae, Hydrophyllaceae, Polemoniaceae, Asclepiadaceae, Primulaceae, Umbelliferae, Begoniaceae, Resedaceae, Cruciferae, Papaveraceae, Portulacaceae, Caryophyllaceae, Amaranthaceae, Chenopodiaceae, and Polygonaceae. Most of the plants to which the disease has been carried are cultivated flowering plants. It has not yet been transmitted to any crop plant of great economic importance. It should be remembered, however, that aster yellows is identical with a rather serious disease of lettuce known in the West as the Rio Grande disease and here in New York State as the white-heart disease of lettuce. It may also cause a serious disease of buckwheat. Yellows can, no doubt, be transmitted to many species of plants that have not been included in my experiments. There are, however, many immune species. It has not been possible to transmit yellows to any plant in the Leguminosae, Rosaceae, or Gramineae.

None of the symptoms of the disease on aster are shown by all species to which yellows has been transmitted. In certain plants it does not cause the production of secondary shoots, clearing of veins, or upright habit of growth. In a few cases it causes little or no chlorosis. There might be some doubt as to whether the disease was actually carried to plants that do not show the symptoms of yellows as they appear on the aster, if it were not



TABLE I. The Host Range of Aster Yellows

Common Name	Scientific Name	Variety	Family	Yellows Transferred	
				From Aster to New Host	To Aster from New Host
China aster	<i>Callistephus chinensis</i> Nees	Many	Compositae	+	+
Shasta daisy	<i>Chrysanthemum maximum</i> Ramond		"	+	
	<i>C. coronarium</i> L.		"	+	
Paris daisy	<i>C. frutescens</i> L.		"	+	+
Ox-eye daisy	<i>C. leucanthemum</i> L.	H 1892	"	+	+
Moon Penny daisy	<i>C. leucanthemum maximum</i>	H 1864	"	+	+
Feverfew	<i>C. sp.</i>	H 1894	"	+	+
Little Gem	<i>C. sp.</i>	H 2765	"	+	+
Feverfew	<i>Matricaria alba</i>	H 3552	"	+	+
	<i>Pyrethrum</i> sp.		"	+	
Swan River daisy	<i>Brachycome iberidifolia</i> Benth.		"	+	
Ammobium	<i>Ammobium alatum</i> R. Br.		"	+	
Gaillardia	<i>Gaillardia aristata</i> Pursh		"	+	
Centaurea	<i>Centaurea margaritae</i> Hort.		"	+	
Centaurea	<i>C. imperialis</i> Hort.		"	+	
	<i>Bellis perennis</i> L.		"	+	
African daisy	<i>Dimorphotheca aurantiacum</i> DC.		"	+	
Salsify	<i>Tragopogon porrifolius</i> L.	Mammoth	"	+	+
Calendula	<i>Calendula officinalis</i> L.		"	+	+
African marigold	<i>Tagetes erecta</i> L.		"	+	+
Strawflower	<i>Helichrysium arenarium</i> DC.		"	+	+
Lettuce	<i>Lactuca sativa</i> L.		"	+	+
Dandelion	<i>Taraxacum officinale</i> Weber		"	+	+
	<i>Calliopsis</i>		"	+	+
	<i>Cosmidium</i>	H 1602	"	+	+
Daisy fleabane	<i>Erigeron annuus</i> (L.) Pers.	H 1898	"	+	+
Butterweed	<i>E. canadensis</i> L.		"	+	+
Horseweed	<i>Ambrosia trifida</i> L.		"	+	+
Hogweed	<i>A. artemisiifolia</i> L.		"	+	+
Sonchus	<i>Sonchus arvensis</i> L.		"	+	+
	<i>S. oleraceus</i> L.		"	+	+
	<i>Scabiosa atropurpurea</i> L.		"	+	+
	<i>Plantago major</i> L.		"	+	+
	<i>Martynia</i>	H 532	Dipsacae	+	+
	<i>Gloxinia</i>	H 2300	Plantaginaceae	+	+
			Martyniaceae	+	+
			Gesneriaceae	+	+



TABLE I (continued)

Common Name	Scientific Name	Variety	Family	Yellows Transferred	
				From Aster to New Host	To Aster from New Host
Monkey flower	<i>Mimulus luteus</i> L.	H 3030	Scrophulariaceae	+	+
	Nemesia	H 1380	"	+	
Summer savory Cultivated lavender Forget-me-not	Calceolaria	H 3675	Solanaceae	+	+
	Schizanthus	H 3602	"	+	
	Salpiglossis		Labiatae	+	
	<i>Satureia hortensis</i> L.	H 977	"	+	
	Lavender		Boraginaceae	+	
Dill Anise	<i>Myosotis scorpioides</i> L.	H 3040	Hydrophyllaceae	+	+
	Nemophila		Polemoniaceae	+	
	<i>Phlox drummondii</i> Hook.		"	+	
	<i>P. paniculata</i> L.		Asclepiadaceae	+	
	<i>Asclepias nivea</i> L.		Primulaceae	+	
	<i>Primula elatior</i> Hill		Umbelliferae	+	
	<i>Didascus caeruleus</i>	H 2155	"	+	+
	<i>Anethum graveolens</i> L.	H 951	"	+	
	<i>Pimpinella Anisum</i>		Begoniaceae	+	+
	<i>Begonia semperflorens</i> Link and Otto		Resedaceae	+	
Mignonette Sweet alyssum California poppy	<i>Reseda odorata</i> L.	H 1052	Cruciferae	+	
	<i>Alyssum compactum procumbens</i>		Papaveraceae	+	
	<i>Eschscholzia californica</i> Cham.		Portulacaceae	+	
	<i>Calandrinia grandiflora</i> Lindl.		"	+	
	Portulaca	H 3500	Caryophyllaceae	+	+
Spinach Buckwheat	<i>Gypsophila paniculata</i> L.		"	+	
	<i>Silene pendula</i> L.	H 2110	"	+	
	Dianthus		Amaranthaceae	+	
	<i>Amaranthus caudatus</i> L.		"	+	
	<i>A. auroro</i>	H 1073	Chenopodiaceae	+	
	<i>Spinacia oleracea</i> L.		Polygonaceae	+	
	<i>Fagopyrum esculentum</i> Moench			+	

Some of the plants to which aster yellows has been transmitted are shown in the table. Each number preceded by the letter *H* in the column showing the variety indicates that the plant was obtained from seed listed under that number in the 1924 seed catalog of Peter Henderson and Company. The last column shows whether the disease was carried to or from aster from or to each of the new hosts.

for the fact that it was carried from most of the plants back to aster and that the disease so transmitted showed all the symptoms of yellows. Many host plants, however, show symptoms typical of yellows on aster. The most striking symptoms shown by diseased specimens of each of the new host plants listed in table 1 are given below.

*Chrysanthemum maximum* Ramond (Shasta daisy). Plants show general chlorosis and upright habit of growth. Clearing of veins appears in some leaves. Most specimens are only partly diseased.

*C. coronarium* L. Plants are chlorotic and produce many secondary shoots. Petals of ray flowers are much reduced in size and are often a green color.

*C. frutescens* L. (Paris daisy). Plants are dwarfed and chlorotic, as shown in figure A, Plate XLI. Secondary shoots show upright habit of growth. Petals of ray flowers are much reduced in size and are more or less green in color.

*C. leucanthemum* L. (Ox-eye daisy). Plants usually show one-sided infection, chlorosis, and upright habit of growth. Ray flowers are dwarfed and green.

*C. leucanthemum maximum* (Moon Penny daisy, H 1892). Except for slight clearing of veins, the diseased leaves show practically no chlorosis. They are not more erect than normal ones. No extra secondary shoots are produced. This host shows a high degree of tolerance for the disease.

*Chrysanthemum* sp. (Feverfew, H 1864). Plants show chlorosis, upright habit of growth, and many secondary shoots.

*Chrysanthemum* sp. (Little Gem, H 1894). Plants are chlorotic and produce many secondary shoots.

*Matricaria alba* (Feverfew, H 2765). Plants are dwarfed and chlorotic, as shown in figure C, Plate XLI. Many secondary shoots are produced.

*Pyrethrum* sp. (H 3552). Plants are dwarfed and chlorotic. They produce many upright-growing secondary shoots.

*Brachycome iberidifolia* Benth. (Swan River daisy). Plants are dwarfed and chlorotic; secondary shoots are very thin, and leaves are reduced in size.

*Ammobium alatum* R. Br. Plants are dwarfed and chlorotic. They remain in the rosette stage and produce many side shoots. The leaves show bronzing, are turgid and much more brittle than normal ones. They have wavy margins and often turn bottom-side up, as is shown in figure A, Plate XLIII.

*Gaillardia aristata* Pursh. Plants badly dwarfed and chlorotic, with many side shoots, bearing small upright-growing leaves as shown in figure F, Plate XLI.

*Centaurea margaritae* Hort. Plants are stunted and very chlorotic. Numerous side shoots are produced, as shown in figure E, Plate XLI. Stem tissues are chlorotic and show a reddish tinge of color. Some diseased leaves are much longer than normal leaves of the same age.

*C. imperialis* Hort. Plants are stunted and chlorotic, as shown in figure I, Plate XLI. Many upright secondary shoots are produced.

*Bellis perennis* L. Plants are dwarfed and chlorotic. The leaves show a reddish tinge of color along the veins. Leaves are not more erect than those on healthy plants. Secondary shoots die while small.

*Dimorphotheca aurantiacum* DC. (African daisy). Plants are dwarfed and produce many secondary shoots, as shown in figure D, Plate XLI. The leaves and side shoots are very chlorotic. Some of the diseased leaves are very narrow and consist of little more than a midrib. Plants are very chlorotic. The ray flowers are dwarfed and green in color. Many flowers are elongated as they are in the aster. The flower heads are dwarfed.

*Tragopogon porrifolius* L. (Salsify). Plants are dwarfed and chlorotic, as shown in figure G, Plate XLI. Many small secondary shoots are produced. Chlorosis in the leaves of this plant sometimes appears in the form of a pattern composed of areas of tissue of light- and dark-green color.

*Calendula officinalis* L. Plants are dwarfed and chlorotic. The flowers are affected in much the same ways as are those of the aster.

*Tagetes erecta* L. (African marigold). Plants are dwarfed and chlorotic, as shown in figure F, Plate XLII. Secondary shoots develop earlier than in normal plants but are not more numerous. Plants never become bushy, and the habit of growth is not noticeably changed. Plants that are infected when young fail to blossom.

*Helichrysum arenarium* DC. (Strawflower). Plants are dwarfed and chlorotic. They produce many secondary shoots. The flowers are affected very much as are aster flowers.

*Lactuca sativa* L. (Lettuce). Plants are dwarfed and chlorotic. They fail to make heads, but produce many upright secondary shoots. The margins of diseased leaves often show brown-colored pustules which are old latex clots. Flowering side branches are greatly shortened, as shown in figure C, Plate XLIII. Figure F, Plate XLIII, shows the effect of the disease on romaine. The chlorosis of romaine described by Clinton (4) was probably caused by the aster yellows disease.

*Taraxacum officinale* Weber (Dandelion). Plants are chlorotic and produce numerous secondary shoots. The leaves are reduced in width, show clearing of veins and a more upright habit of growth than do the leaves of normal plants. This is shown in figure H, Plate XLI. One of the most striking symptoms on this plant is the bronzing or reddening of the leaves. The flower heads are affected much as they are in the aster.

Calliopsis (H 1602). Plants are dwarfed and chlorotic and produce many slender secondary shoots.

Cosmidium (H 1898). Plants are dwarfed and chlorotic. Many secondary shoots are produced.

*Erigeron annuus* (L.) Pers. (Daisy fleabane) and *E. canadensis* L. (Butterweed). The symptoms on these two species are essentially alike.

Plants show a great bunching of leaves due to shortening of internodes. They produce large numbers of upright secondary shoots. Leaves are chlorotic and reduced in size. Flowers are affected much as are those of the aster. A diseased and a healthy plant of *E. annuus* are shown in figure B, Plate XLIII.

*Ambrosia trifida* L. (Horseweed). Plants are somewhat dwarfed, as shown in figure E, Plate XLII. Leaves show clearing of veins and are chlorotic while young. Old leaves become a yellowish green color.

*A. artemisiifolia* L. (Hogweed). Plants are stunted and quite chlorotic. Many plants show one-sided infection.

*Sonchus arvensis* L. and *S. oleraceus* L. (Sow thistle). Plants are much stunted and chlorotic. Internodes of stems are shortened, and numerous secondary shoots are produced. Leaves show clearing of veins and are less serrate than normal leaves. Most plants do not flower. A diseased and a healthy plant of *S. oleraceus* are shown in figure A, Plate XLII.

*Scabiosa atropurpurea* L. Plants are stunted and chlorotic. They produce large numbers of secondary shoots.

*Plantago major* L. Plants are chlorotic. Leaves show clearing of veins and upright habit of growth. The petioles are abnormally elongated.

*Martynia* (H 532). Plants are chlorotic and produce numerous secondary shoots.

*Gloxinia* (H 2300). Plants produce many secondary shoots and do not blossom. They are slightly chlorotic.

*Mimulus luteus* L. (Monkey flower). Plants are somewhat dwarfed and slightly chlorotic. The stems are not more than half as thick as those of healthy plants. Many slender secondary shoots are produced. Large numbers of aerial roots grow from the nodes.

*Nemesia* (H 3030). Plants are dwarfed and chlorotic.

*Calceolaria* (H 1580). Plants are chlorotic and much dwarfed. Secondary shoots remain small, and leaves are much reduced in size.

*Schizanthus* (H 3675). Plants are slightly chlorotic with numerous fine secondary shoots, as shown in figure G, Plate XLII. Leaves are much reduced in size.

*Salpiglossis* (H 3602). Plants are much dwarfed and chlorotic.

*Satureia hortensis* L. (Summer savory). Plants are somewhat dwarfed and slightly chlorotic. Numerous secondary shoots show an upright habit of growth.

Lavender (H 977). Plants are stunted and slightly chlorotic. Numerous secondary shoots are produced.

*Myosotis scorpioides* L. Plants are slightly chlorotic and somewhat stunted. They produce numerous upright slender secondary shoots. The leaves are much reduced in size.

*Nemophila* (H 3040). Plants are much dwarfed. The tips of diseased branches are almost white. Numerous short secondary shoots are produced.

Flowers show great variation in size; some are minute. Many of the flowers are of a green color.

*Phlox drummondii* Hook. and *P. paniculata* L. Plants are stunted and chlorotic. The flowers are reduced in size. Many of them are green. Secondary flowers are sometimes produced on the stigmas of other flowers. Such flowers may in turn bear flowers on their stigmas.

*Asclepias nivea* L. Leaves are narrow and yellowish green. They frequently show clearing of veins. A diseased and a healthy plant are shown in figure *E*, Plate XLIII.

*Primula elatior* Hill. Plants are stunted and chlorotic. They produce many secondary shoots. The leaves are greatly reduced in size.

*Didiscus caeruleus* (H 2155). Plants are stunted and chlorotic.

*Anethum graveolens* L. (Dill). Plants are dwarfed and chlorotic, as shown in figure *B*, Plate XLI. They do not produce secondary shoots. Leaves are not more upright in habit of growth than are those of healthy plants.

*Pimpinella Anisum* (Anise, H 951). Plants are stunted and slightly chlorotic. They produce many fine secondary shoots. The leaves are reduced in size. A diseased and a healthy plant are shown in figure *D*, Plate XLII.

*Begonia semperflorens* Link and Otto. Plants are somewhat dwarfed and slightly chlorotic. The leaves are reduced in size and show clearing of veins. Some secondary shoots are produced.

*Reseda odorata* L. (Mignonette). Plants are slightly chlorotic and somewhat dwarfed, as shown in figure *J*, Plate XLI. The most striking symptom is the bronzing and reddening of diseased leaves. The secondary shoots show an upright habit of growth. Secondary flowers are produced on the stigmas of other flowers, as shown in figure *K*, Plate XLI.

*Alyssum compactum procumbens* (Sweet alyssum, H 1052). Plants produce thin chlorotic secondary shoots.

*Radicula palustris* var. *hispida* (Desv.) Robinson. Plants are greatly dwarfed and produce numerous secondary shoots, but show no chlorosis.

*Eschscholtzia californica* Cham. Plants are dwarfed and chlorotic. Many secondary shoots are produced.

*Calandrina grandiflora* Lindl. Plants are stunted and chlorotic. They produce many secondary shoots, as is shown in figure *H*, Plate XLII. A healthy plant is pictured in figure *I*, Plate XLII.

*Portulaca* (H 3500). Plants are dwarfed and chlorotic. Many secondary shoots are produced.

*Gypsophila paniculata* L. Plants are dwarfed and produce many secondary shoots, as shown in figure *B*, Plate XLII. Leaves are chlorotic and show an upright habit of growth. They are often longer than the leaves of healthy plants.

*Silene pendula* L. Plants are stunted and somewhat chlorotic. The leaves are very narrow.

*Dianthus* (H 2110). Plants are stunted and chlorotic.

*Amaranthus caudatus* L. and *A. auroro* (H 1073). Plants are stunted and chlorotic. Numerous short secondary shoots are produced. The leaves show clearing of veins. The disease causes the leaves of *A. auroro*, which are normally red, to become yellowish gray. A diseased and a healthy plant of *A. auroro* are shown in figure F, Plate XLIII.

*Spinacia oleracea* L. (Spinach). Plants are dwarfed and slightly chlorotic. Many upright secondary shoots are sometimes produced. The leaves show clearing of the veins.

*Fagopyrum esculentum* Moench (Buckwheat). Plants are slightly chlorotic and somewhat dwarfed, as shown in figure C, Plate XLII. The diseased plants appear much like healthy ones except that many of the flowers are green and occur in large bunches. An indefinite number of flowers are produced in the leaf axils.

TABLE 2. *The Transfer of Yellows from Various Hosts to Aster*

		Exposed April 24-April 30	Exposed April 30-May 5	Exposed May 5-May 15	Exposed May 15-May 25	Exposed May 25-May 30	No. of Insects Alive May 30
China aster.....	Diseased specimen	+	+	+	+	+	23
	Healthy specimen	++	++	++	++	++	14
Moon Penny daisy.....	Diseased specimen	+	+	+	+	+	20
	Healthy specimen	+	+	+	+	+	13
Little Gem chrysanthemum.....	Diseased specimen	+	+	+	+	+	21
	Healthy specimen	+	+	+	+	+	14
<i>Centaurea imperialis</i> .....	Diseased specimen	+	+	+	+	+	23
	Healthy specimen	+	+	+	+	+	22
<i>Silene pendula</i> .....	Diseased specimen	+	+	+	+	+	10
	Healthy specimen	+	+	+	+	+	1
Calliopsis.....	Diseased specimen	+	+	+	+	+	20
	Healthy specimen	+	+	+	+	+	15
<i>Matricaria alba</i> .....	Diseased specimen	+	+	+	+	+	12
	Healthy specimen	+	+	+	+	+	20
Salpiglossis.....	Diseased specimen	+	+	+	+	+	2
	Healthy specimen	+	+	+	+	+	2
<i>Gaillardia aristata</i> .....	Diseased specimen	+	+	+	+	+	13
	Healthy specimen	+	+	+	+	+	15
Salsify.....	Diseased specimen	+	+	+	+	+	8
	Healthy specimen	+	+	+	+	+	7
Schizanthus.....	Diseased specimen	+	+	+	+	+	2
	Healthy specimen	+	+	+	+	+	1
<i>Amaranthus auroro</i> .....	Diseased specimen	+	+	+	+	+	8
	Healthy specimen	+	+	+	+	+	9
<i>Gypsophila paniculata</i> .....	Diseased specimen	+	+	+	+	+	4
	Healthy specimen	+	+	+	+	+	6
<i>Primula elatior</i> .....	Diseased specimen	+	+	+	+	+	10
	Healthy specimen	+	+	+	+	+	12
<i>Ammobium alatum</i> .....	Diseased specimen	+	+	+	*	+	15
	Healthy specimen	+	+	+	+	+	8

TABLE 2 (continued)

		Exposed April 24-April 30	Exposed April 30-May 5	Exposed May 5-May 15	Exposed May 15-May 25	Exposed May 25-May 30	No. of Insects Alive May 30
<i>Myosotis scorpioides</i> . . . . .	Diseased specimen	+	+	+	+	+	4
	Healthy specimen	+	+	+	+	+	7
<i>Dimorphotheca aurantiaca</i> . . . . .	Diseased specimen	+	+	+	+	+	19
	Healthy specimen	+	+	+	+	+	6
<i>Nemophila</i> . . . . .	Diseased specimen	+	+	+	+	+	9
	Healthy specimen	+	+	+	+	+	10
<i>Mimulus luteus</i> . . . . .	Diseased specimen	+	+	+	+	+	6
	Healthy specimen	+	+	+	+	+	9
<i>Bellis perennis</i> . . . . .	Diseased specimen	+	+	+	+	+	6
	Healthy specimen	+	+	+	+	+	23
<i>Anethum graveolens</i> . . . . .	Diseased specimen	+	+	+	+	+	17
	Healthy specimen	+	+	+	+	+	6
<i>Brachycome iberidifolia</i> . . . . .	Diseased specimen	*	+	+	+	+	14
	Healthy specimen	+	+	+	+	+	10
<i>Chrysanthemum frutescens</i> . . . . .	Diseased specimen	+	+	+	+	+	11
	Healthy specimen	+	+	+	+	*	15
<i>Scabiosa atropurpurea</i> . . . . .	Diseased specimen	+	+	+	+	+	8
	Healthy specimen	+	+	+	+	+	6
<i>Sonchus arvensis</i> . . . . .	Diseased specimen	+	+	+	+	+	14
	Healthy specimen	+	+	+	+	+	14
<i>Erigeron annuus</i> . . . . .	Diseased specimen	+	+	+	+	+	10
	Healthy specimen	+	+	+	+	+	6
<i>Taraxacum officinale</i> . . . . .	Diseased specimen	+	+	+	+	+	20
	Healthy specimen	+	+	+	+	+	16
<i>Ambrosia trifida</i> . . . . .	Diseased specimen	+	+	+	+	+	11
	Healthy specimen	+	+	+	+	+	18

The first column of the table gives the names of the different plants from which yellows was transferred back to asters. A healthy specimen served in each case as a check on the diseased specimen. The dates given at the top of the table show the periods of time during which the five sets of aster plants were exposed. The figures in the last column show the number of insects alive in each culture when the experiment was ended. A plus sign (+) indicates a healthy aster plant; a double plus sign (++) a yellowed plant, and an asterisk (\*) a dead plant.

#### THE TRANSFER OF ASTER YELLOWS FROM VARIOUS HOST PLANTS BACK TO THE ASTER

The transfer of yellows from various host plants to which it was experimentally transmitted back to the aster was described under the section on host range of the disease. One of the experiments in which the disease was taken to aster from several different hosts to which it had been transmitted from aster is given here.

On April 16, 1925, 25 virus-free nymphs in the second, third, and fourth instars were transferred to a yellowed and to a healthy plant respectively of each of the species listed in table 2. The insects were kept on these plants for 8 days, from April 16 to April 24. Each colony was then transferred to a succession of healthy young aster plants. The first set of plants was exposed for 6 days; the second set for 5 days; the third set for 10 days;

the fourth set for 10 days; and the fifth set for 5 days. The results obtained are shown in table 2. A plus sign indicates that the plant remained healthy, a double plus sign that it was diseased, and an asterisk that it died before the transmission record was obtained. The figures given in the last column of the table show the number of insects alive in each colony when the experiment was ended. All check plants remained healthy.

With the exception of the two plants exposed to insects from the diseased aster and the diseased Scabiosa plant, all the aster plants of the first set remained healthy. The plant exposed to insects from the yellowed specimen of *Brachycome iberidifolia* died before its transmission record was obtained. All plants of the second set remained healthy except those exposed to insects from the diseased specimens of aster and Scabiosa. All plants of the third set exposed to insects from the diseased specimens became diseased except those exposed to insects from *Matricaria alba*, *Gypsophila paniculata*, *Primula elatior*, *Brachycome iberidifolia*, *Amaranthus auroro*, and Schizanthus. All plants of the fourth set exposed to insects from diseased specimens became diseased except those exposed to insects from diseased specimens of *Ammobium alatum*, *Primula elatior*, *Amaranthus auroro*, and Schizanthus. The plant exposed to insects from *Ammobium* died prematurely. All plants of the fifth set exposed to insects from diseased specimens became diseased except those exposed to insects from *Primula elatior*, *Amaranthus auroro*, and Schizanthus. The check plant exposed to insects from the healthy specimen of *Chrysanthemum frutescens* died prematurely.

It will be seen that yellows was transmitted to aster from all the different plants listed in the table except *Amaranthus auroro*, *Primula elatior*, and Schizanthus. It is apparently difficult for the insects to obtain the virus from these plants. The colonies of insects from Schizanthus were very small, indicating that this plant is not a suitable host for *Cicadula sexnotata*. The insects seem to do well on *Primula elatior*, but it is a plant which they avoid when other suitable host plants are available. *Amaranthus auroro* is, however, a favorite host plant for the insect. The failure of *Cicadula* to transmit yellows from these three plants back to aster is not considered to be evidence that the plants did not have the aster yellows disease. Yellows was repeatedly transmitted to all these hosts. That the disease was transmitted back to aster from the other 25 hosts proves that the symptoms observed on these plants were those of aster yellows. The failure of many of the colonies of insects from diseased specimens to transmit yellows to plants of the first and second sets shows that the incubation period of the virus in the insects of these colonies was not completed during the time the plants were exposed. The plants belonging to the first set exposed to insects from diseased specimens of aster and Scabiosa became diseased. This is thought to indicate that the disease is obtained very readily from these plants and that the insects picked up the virus soon after being placed on them. No significant variations were observed in the symptoms or the severity of the



disease carried back to aster from the different host plants shown in the table. Particular attention was given to this point as it was thought that passage through some of these hosts might attenuate the virus and might give rise to weak strains of the yellows disease. No evidence that the virus can be attenuated in this way was obtained in this or in other similar experiments.

#### THE TRANSMISSION OF ASTER YELLOWS THROUGH OLD AND YOUNG LEAVES

Ten adult aster leafhoppers hatched and reared on a yellowed aster plant were confined on a small area of a single old leaf on each of 11 different aster plants. They were confined on the leaves by means of small cages made of glass tubes  $1\frac{1}{2}$  cm. in diameter, open at both ends and fitted into holes in cork stoppers held in place by means of pins stuck into other stoppers placed on the surface of the soil, beneath the leaf to be exposed. The upper end of each cage was covered with cheesecloth. The leaf area to be exposed covered the other end. The cage was held in place by the corks. The insects were confined near the distal ends of the leaves. Five healthy aster plants served as checks. The insects were kept on the leaves for 8 days. They were then removed and the plants were fumigated. Twenty days after the insects were removed, one of the plants showed the first symptoms of yellows. Two days later 4 other plants showed the disease, and still later 4 of the remaining 6 became yellowed. The leaves on which the insects were confined never showed any of the symptoms of yellows. Two of the plants that were exposed as well as the 5 check plants remained healthy. It is interesting to note that 5 of the 9 plants that became diseased showed one-sided infection. In each case of one-sided infection the side of the plant on which the exposed leaf was attached became diseased while the opposite side remained healthy. This suggests that the one-sided infection observed in the field takes place through old leaves.

By means of the same small cages 10 virus-carrying adult aster leafhoppers were confined on a small leaf area near the tip of a single young leaf on each of 6 healthy young aster plants. Two other healthy young plants served as checks. The insects were kept on the leaves for 8 days. They were then removed and the plants were fumigated. Fourteen days after the removal of the insects 3 of the plants showed yellows. Two days later the other 3 plants became diseased. None of these plants showed one-sided infection. The check plants remained healthy.

These experiments prove that yellows can be transmitted through both old and young leaves. They also show that transmission through young leaves produces systemic infection while transmission through old leaves sometimes produces one-sided infection.

## RELATION OF ASTER YELLOWS TO OTHER SIMILAR DISEASES

## The Curly-Top Disease of Beets

The writer's first observations on the symptoms of aster yellows led to the realization that this disease is in many respects similar to the curly-top of beets. It was even suspected that the two diseases might be identical in spite of the fact that curly-top does not cause conspicuous chlorosis. Several experiments were made to test the possibility of these diseases being identical.

On February 4, 1924, 150 adult aster leafhoppers hatched and reared on yellowed aster plants were placed in each of 5 cages containing one healthy aster plant and one each of the following varieties of beet plants: Lanes' Improved White sugar beet, Imperial White sugar beet, Klein Wanzleben sugar beet, and Detroit Dark Red garden beet. One healthy aster plant and one plant of each of the beet varieties were kept free of insects and served as checks. The insects were confined on the plants for 3 weeks, after which time they were removed and the plants were fumigated. All the 5 aster plants exposed to virus-carrying insects became diseased. All beet plants exposed to the same insects and all check plants remained healthy. The experiment proves that *Cicadula sexnotata* is unable to transfer yellows to the varieties of beet plants tested, under conditions favorable for the transfer of yellows to aster plants.

On March 27, 1924, 100 adult virus-free aster leafhoppers were placed on 2 sugar beet plants having curly-top. The diseased sugar beet plants were obtained through the kindness of Mr. Edward A. Schwing of the Spreckels Sugar Company, Spreckels, California. After being confined on the curly-top beet plants for 6 days, they were transferred to a cage containing 2 healthy aster plants and 2 healthy plants of each of the varieties of beets used in the previous experiment. The insects were kept on these plants for 3 weeks. They were then removed and the plants were fumigated. All plants remained healthy during 2 months that they were kept under observation. The experiment proves that under conditions favorable for the transfer of aster yellows the aster leafhopper is unable to transmit curly-top to the varieties of beet plants used or to aster plants.

On October 22, 1925, 154 sugar beet leafhoppers were received from Mr. Eubanks Carsner of Riverside, California. Fifty of the insects obtained in this shipment were placed in a cage containing 2 healthy aster plants and 2 healthy plants of each of the varieties of beets used in the above-described experiments. They were confined on these plants for 21 days. At the end of this time all the beet plants had curly-top disease, but both aster plants were still healthy. The aster plants remained healthy during 2 months that they were kept under observation. The experiment shows that virus-carrying beet leafhoppers do not transmit curly-top to aster plants under conditions favorable for the transfer of this disease to beets.

It was observed that the sugar beet leafhoppers prefer beet plants to aster plants, and it was thought that their failure to transfer curly-top to asters in the above-described experiment might be due to their not feeding sufficiently on these plants. A further experiment was made to test this hypothesis. On November 11, 1925, 10 virus-carrying adult beet leafhoppers were confined on a healthy young aster plant. They were left on the plant until November 30, when it was found that only one of the original 10 insects remained alive. The aster plant was still healthy after 2 months, when the experiment was ended. The experiment proves that the sugar beet leafhopper can live on aster plants. It also shows that this leafhopper is unable to transmit curly-top to asters. Whether the curly-top virus was transmitted to aster without causing any symptoms of disease in this plant is not known, since no attempt was made to transmit the virus from such plants to beet plants by means of virus-free beet leafhoppers.

On January 8, 1926, 10 adult beet leafhoppers that had been confined on a yellowed aster plant for 6 days were transferred to a healthy young aster plant. After 18 days only one of the insects remained alive. On January 26, 10 other beet leafhoppers that had been confined on a yellowed aster plant for 10 days were placed on a healthy young aster plant. After 6 days the insects were removed. Both the aster plants exposed to beet leafhoppers that had fed on yellowed aster plants remained healthy during the 2 months that they were kept under observation. The experiment indicates that the beet leafhopper is unable to transmit the aster yellows disease to aster plants.

The above-outlined experiments bring evidence that *Cicadula sexnotata* can not transmit aster yellows to sugar or garden beets and that it can not transmit the curly-top disease to beets or to aster plants. They also show that *Eutettix tenellus* Baker is unable to transmit the curly-top disease to aster plants under conditions favorable for the transfer of this disease to beets, and that it is unable to transmit aster yellows from diseased to healthy aster plants. This evidence, together with the fact that the aster yellows disease and the curly-top disease differ in their plant host ranges and in certain of the symptoms they produce on their respective hosts, leads to the conclusion that the two diseases, though similar in many respects, are quite distinct.

### The Yellows Disease of the Peach

Peach yellows resembles aster yellows in the stimulation of secondary shoots and in causing these shoots to assume an upright habit of growth. Both cause chlorosis and premature death of diseased plants. Both frequently cause one-sided infection. Peach yellows, however, is not known to cause clearing of veins, a symptom which is characteristic of aster yellows on many different hosts. It also spreads more slowly in the field and has a much more limited geographical distribution than does aster yellows. Aster yellows is a common disease in California and in some other western

states where peach yellows is not known. In spite of these differences it was thought that the two diseases might be identical, *i.e.*, due to the same causative agent.

A number of attempts were therefore made to transfer aster yellows to healthy young seedling peach trees. Since all these attempts resulted in failure, only one experiment will be described in detail in order to illustrate the methods used.

On September 20, 1924, 25 adults of *Cicadula sexnotata*, hatched and reared on yellowed aster plants, were transferred to each of 20 young peach seedlings growing in pots. Fifty similar adults were transferred to each of 8 other healthy young peach seedlings, while 8 additional healthy seedlings of the same age served as checks. After 10 days all the insects confined on these plants were dead. *C. sexnotata* can not live indefinitely on the peach. None of the peach seedlings showed symptoms of yellows during the 2 years they were kept under observation.

Attempts were also made to transfer peach yellows to aster plants by means of *C. sexnotata*. During the summer of 1924, buds from a yellowed Elberta peach tree were budded into healthy young seedlings. In the spring of 1925 the seedlings were cut back and the buds were forced in the usual way. The diseased buds grew into small trees during the summer. Some of these trees brought into a greenhouse in the autumn produced the secondary shoots and yellowed foliage typical of peach yellows. On January 20, 1926, 312 virus-free nymphs of *C. sexnotata* in the first, second, and third instars were confined on one of these yellowed peach trees. After 5 days only 27 of the insects remained alive. They were transferred to a succession of healthy young aster plants as follows: aster number 1 was exposed from Jan. 25 to Feb. 2; aster number 2 from Feb. 2 to Feb. 8; aster number 3 from Feb. 8 to Feb. 13; aster number 4 from Feb. 13 to Feb. 20; aster number 5 from Feb. 20 to Feb. 25; aster number 6 from Feb. 25 to Mar. 1; aster number 7 from Mar. 1 to Mar. 9; aster number 8 from Mar. 9 to Mar. 25; aster number 9 from Mar. 25 to Mar. 28; aster number 10 from Mar. 28 to Mar. 31; and aster number 11 from Mar. 31 to Apr. 5. On March 9, 18 of the original 27 insects remained alive. On April 5, when the last exposure was completed, only one insect was living. All the aster plants remained healthy during the 2 months they were kept under observation. The experiment shows that *Cicadula* can not transmit peach yellows from peach to aster.

Attempts were also made to transfer peach yellows to healthy peach seedlings by means of *C. sexnotata*, but only negative results were obtained.

The experiments described above indicate that *Cicadula* is unable to transfer aster yellows to the peach or peach yellows to peach or to aster. They also bring evidence that aster yellows and peach yellows are distinct diseases. They do not, however, preclude the possibility that some other insect species might be able to transfer aster yellows to the peach.

### The Stunt Disease of Dahlia

Since aster yellows attacks many Composites it was thought that it might go to the Dahlia and that it might be identical with the well known stunt disease of this plant. Attempts were, therefore, made to transmit aster yellows to Dahlia plants by means of *Cicadula sexnotata*. Dahlia seed of the variety Collarette listed in Henderson's seed catalog for 1926 under the number 2072 was planted in flats in a greenhouse. Potted seedlings of this variety were used in all experiments described below.

On March 3, 1926, 100 virus-carrying adults of *Cicadula sexnotata* were placed on each of 2 healthy young Dahlia plants. Two other plants of the same age and variety not exposed to insects served as checks. The insects were confined on the plants for 7 days. They were then removed. The plants were fumigated and placed in an insect-proof cage, where they were kept under observation for 3 months. The 2 plants exposed to insects remained small and produced many secondary shoots, while the check plants grew normally. In order to determine whether the stunted condition of these plants was due to the presence of the aster yellows virus the following experiment was made.

On April 19, 1926, 150 virus-free nymphs of *C. sexnotata* in the third, fourth, and fifth instars were confined on one of the stunted plants exposed to insects in the first experiment. The same number of virus-free nymphs in similar stages of development were confined on one of the check plants. The insects were kept on these plants for one week. Thirty insects from each culture were then placed on each of 4 healthy young aster plants. Thirty adults from a yellowed aster plant placed on another healthy young aster served as a check. These 9 insect cultures were transferred to a succession of healthy young aster plants as follows: the nine plants of the first series were exposed from April 26 to April 28; those of the second series from April 28 to April 30; those of the third series from April 30 to May 4; those of the fourth series from May 4 to May 7; those of the fifth series from May 7 to May 10; and those of the sixth series from May 10 to May 14. The 24 aster plants exposed to insects from the stunted Dahlia plant as well as the 24 aster plants exposed to insects from the normal Dahlia plant remained healthy during 2 months that they were kept under observation. The 6 plants exposed to the culture of insects from the yellowed aster plant all became diseased. The experiment proves that the insects were unable to obtain the aster yellows virus from the stunted Dahlia plant. They did not spread the disease under conditions favorable for its transmission. It also brings evidence that the stunting produced in the first experiment was due to insect injury and not to the aster yellows disease.

In order to test further this conclusion, an experiment in which Dahlia seedlings were exposed to small numbers of virus-carrying and virus-free insects was undertaken. Three insect colonies consisting of 30 virus-carrying adults each and one colony of insects consisting of 30 virus-free

adults were transferred to a succession of healthy young Dahlia plants. The first set of plants were exposed to these colonies from May 21 to May 28; the second set from May 28 to June 1; the third set from June 1 to June 4; the fourth set from June 4 to June 7; the fifth set from June 7 to June 11. Five Dahlia seedlings of the same age and variety served as checks. All plants were kept under observation for 2 months. The plants exposed to virus-carrying insects grew as fast and were as free from secondary shoots as those exposed to virus-free insects. The plants exposed to virus-carrying as well as those exposed to virus-free insects remained normal and similar to the check plants. The experiment proves that when Dahlia seedlings are exposed to only 30 virus-bearing insects for a few days they not only remain free from yellows but suffer no noticeable effects from the feeding of the insects.

The experiments with Dahlia seedlings prove that *Cicadula sexnotata* does not transfer aster yellows to Dahlia plants under conditions favorable for the transmission of the disease. All evidence obtained indicates that the disease known as Dahlia stunt is distinct from aster yellows. Severe injury to Dahlia seedlings caused by the feeding of large numbers of *C. sexnotata* produces stunting and growth of secondary shoots. Such plants resemble those having stunt disease, but it is not considered that stunt disease is in any way related to the feeding of this leafhopper, since *C. sexnotata* does not occur in large numbers on Dahlia plants.

### Yellows Disease of the Strawberry

In the autumn of 1924, Mr. George M. Darrow of the U. S. Department of Agriculture kindly sent the writer several specimens of yellowed strawberry plants. The plants were placed in a garden near a bed of healthy strawberry plants. During the summer of 1925 it was observed that the yellows spread in some way to previously healthy plants. This yellows disease of the strawberry does not closely resemble aster yellows. Nevertheless, attempts were made to transmit aster yellows to strawberry plants. Thirty virus-carrying adults of *Cicadula sexnotata* hatched and reared on yellowed aster plants were confined on 4 healthy strawberry plants for 5 days. They were then removed and the plants were fumigated. Three other healthy strawberry plants were exposed in a cage containing several hundred virus-carrying leafhoppers for 2 weeks. The plants were then removed and fumigated. Two plants not exposed to insects served as checks. All plants remained healthy during 3 months that they were kept under observation. This experiment brings evidence that aster yellows can not be transmitted to the strawberry by *C. sexnotata* and that it is not identical with the yellows disease observed on the strawberry. Whether this yellows is identical with the strawberry yellows recently described by Plakidas (22) is not known.

### False Blossom of the Cranberry

Some of the symptoms of aster yellows are similar to those associated with the false-blossom disease of the cranberry. It was thought that the two diseases might be identical. Several attempts were made to transmit aster yellows to healthy young cranberry seedlings by means of *Cicadula sexnotata*. Since all these attempts resulted in failure, only one experiment will be described in detail.

On September 20, 1924, 6 healthy cranberry plants were placed in a large cage containing several hundred virus-carrying adults of *C. sexnotata*. They were left in the cage for 2 weeks, after which time they were removed and fumigated. Two similar plants not exposed to insects served as checks. All plants remained healthy during 6 months that they were kept under observation.

Since it was not possible to transmit aster yellows to the cranberry by means of *C. sexnotata*, it is concluded that this disease is distinct from false-blossom.

### FAILURE OF MECHANICAL TRANSMISSION

During the past 3 years many attempts have been made to transmit aster yellows mechanically. The following experiments indicate the methods used.

Portions of yellowed plants were crushed and the undiluted juice was taken for use. The juice was transferred by means of absorbent cotton to wounds made in several different ways. Wounds were made in the tissues of the plants to be inoculated, (1) by jabbing a needle into the lower portions of petioles of the youngest mature leaves; (2) by cutting off the youngest mature leaves; (3) by slitting with scissors the youngest leaves large enough to cut; (4) by tearing with forceps leaves of different ages; (5) by jabbing a needle into stems and buds; and (6) by rubbing and crushing young leaves between thumb and finger. Twelve plants were wounded by each of these methods, and a liberal amount of juice from diseased plants was rubbed into the wounds. Twelve unwounded and uninoculated plants served as checks. The plants were kept in insect-proof cages under favorable conditions for growth. All the plants were from seed planted 46 days before the experiment was started. All plants remained healthy for 2 months after they were inoculated, when the experiment was ended. The experiment shows that yellows is not transmitted under the conditions and by the methods used.

In another experiment, juice from diseased leaves was thoroughly rubbed into young leaves wounded by crushing between thumb and finger. One hundred fifty young plants were subjected to this treatment. All the plants remained healthy during 56 days that they were kept under observation.

Diseased leaf tissue from a yellowed aster plant was crushed and forced into small slits made in the petioles of leaves of 12 healthy young aster plants. Twelve similar unwounded plants kept in the same greenhouse



served as checks. All plants remained healthy during the 2 months the experiment was in progress.

At another time crushed leaf tissues from diseased plants were placed in wounds made in stems and leaf petioles of 25 healthy young aster plants. Juice from diseased leaves was passed through filter paper in order to remove bits of tissue, and was then injected by means of a hypodermic needle into the stems and leaf petioles of 20 other healthy young aster plants. Unfiltered juice from yellowed aster plants was rubbed into wounds made on leaves by scratching lightly with a sharp needle. Six needle scratches were made close together and parallel to the long axis of the leaf, and 6 more at right angles to and across the first 6 scratches. Four leaves were wounded on each plant, and 25 plants were subjected to the treatment. Fresh unfiltered juice was applied to wounds made by rubbing young leaves between thumb and finger. Twenty-five healthy young plants were treated in this way. All the plants used in the experiment were kept under observation for 2 months. All remained healthy.

Four hundred adults of *Cicadula sexnotata* reared on yellowed aster plants were confined in a large test tube for one hour. At the end of this period the bottom and sides of the tube were covered with feces. The insects were removed, and a few cubic centimeters of water were added to the tube. The feces were then dislodged and rubbed up in the water by means of a camel's-hair brush. This mixture was rubbed into 60 needle-jab wounds made in the leaves and petioles of each of 50 healthy young aster plants. The plants were kept under observation for 3 months, but all remained healthy.

Juice was extracted from the crushed leaves of 10 different aster plants that had been diseased for varying periods of time. One of the plants showed the earliest recognizable symptoms of yellows. The other 9 plants had been diseased for periods of time varying from 3 days to 6 weeks. The juice from these plants was mixed and rubbed into 60 needle jabs made in the young leaves of each of 25 rapidly growing, healthy young plants. All the plants remained healthy during 2 months that they were kept under observation.

Two hundred adults of *C. sexnotata* hatched and reared on yellowed aster plants were crushed in a mortar and rubbed up in a small amount of water. The mixture was then rubbed into 60 needle-jab wounds made in the young leaves of each of 25 healthy young plants. All plants used in this experiment were kept under observation for 2 months. All remained healthy.

Buds from diseased branches were budded into the main stems of 12 large, healthy aster plants. The buds were inserted near the ground level. They lived in 7 of the 12 plants. The other 5 buds died. The plants in which the buds grew were cut back to within a few inches of the ground level. This was done in order to force the buds into growth. All the diseased buds produced small diseased branches. The buds in the axils of



leaves above the point where the diseased buds were inserted also grew into short branches. In the cases of 3 of the plants, these branches became yellowed. The other 4 plants died before disease was observed in the branches above the point of insertion of the diseased bud. The experiment shows that yellows can be transmitted by budding. All attempts to transfer aster yellows mechanically, except by budding, have failed.

#### YELLOW S NOT TRANSMITTED THROUGH ASTER SEED

Most virus diseases of plants are not readily transmitted through seeds. Smith (25), working with peach pits from yellowed trees, obtained very poor germination. The pits that germinated gave healthy seedlings. Miss Westerdijk (32) claims that tomato mosaic is transmitted through tomato seed, but Dickson (5) and Gardner and Kendrick (9) grew thousands of plants from seed of mosaic tomatoes without getting a single diseased seedling. Wilbrink and Ledebor (33) and others have shown that sugar cane mosaic is not transmitted by sugar cane seed. Brandes and Klaphaak (2) reached the same conclusion regarding its relation to seeds of corn and of certain wild grasses. According to Doolittle and Walker (6), cucumber mosaic is not transmitted by the seeds of cucumber, squash, muskmelon, and pumpkin, but is transmitted by the seeds of the wild cucumber, *Micrampelis lobata*.

The mosaic diseases of leguminous plants, on the other hand, seem to be quite generally transmitted through seed. This was found to occur in the case of the mosaic of pea bean by Reddick and Stewart (23) and for the mosaic of lima bean by McClintock (16). Gardner and Kendrick (8) have shown that mosaic is transmitted by soybean seed, and Dickson (5) has shown that it is transmitted by the seed of *Trifolium pratense*, *T. hybridum*, *Melilotus alba*, and *Pisum sativum*.

Mention has already been made of the fact that yellowed aster plants often show the symptoms of disease on one side only. The same one-sided infection is also shown by many of the diseased flower heads. Seeds were collected from the diseased and healthy flower heads of a number of aster plants showing one-sided infection. Seeds were also collected from flower heads showing one-sided infection. Seeds from wholly diseased flower heads and from diseased parts of partly diseased flower heads do not germinate. During the fall and winter of 1923 approximately 1,200 aster seedlings were grown from seeds from partly diseased aster plants, and 600 seedlings were grown from seeds from partly diseased flower heads. All these seedlings remained healthy during the 2 months that they were kept under observation. During the 2 years that have passed since these experiments were completed, many hundreds of seedlings have been grown from the seeds of partly diseased aster plants, but in no case have yellowed seedlings been obtained. Chlorotic seedlings occasionally appear but die after a few weeks. They are similar to the albinos produced by many

different species of plants. They do not resemble plants having yellows. It seems safe to conclude that aster yellows is very rarely, if ever, transmitted through aster seeds.

#### OVER-WINTERING OF ASTER YELLOWS

All evidence obtained to date indicates that aster yellows is not transmitted through the seeds of aster plants or through the eggs of the carrier insect. As the aster is an annual and the transmitting insect seems to pass the winter in the egg stage only, these two organisms are probably unable to carry it over winter. It is possible that yellows may be transmitted through the seeds of some host plant other than aster. It is also possible that some other insect may be able to transmit and to carry it through the winter. But biennial and perennial host plants furnish the only means by which aster yellows is now known to overwinter.

In the early spring of 1925 several wild plants of *Plantago major* L., *Chrysanthemum leucanthemum* L., *Sonchus arvensis* L., *Poa annua* L., and a perennial species of *Erigeron* suspected of having yellows, were transplanted together with healthy-appearing specimens of the same species from golf links near the Boyce Thompson Institute to pots placed in insect-proof cages in a greenhouse. On June 20 approximately 100 virus-free nymphs were transferred to a yellowed and to a healthy-appearing plant of *Plantago major*. They were allowed to feed on these plants for 4 days. The nymphs were then removed, and each of the two lots was divided into 4 approximately equal lots of about 25 insects each. They were then separately caged on 8 healthy young aster plants. After 10 days the 8 colonies were transferred to 8 other healthy young aster plants. Six days later they were again transferred to another set of healthy young aster plants on which they were allowed to feed for 6 days. All the plants in the first set remained healthy. Three of the plants in the second set, and the 3 corresponding plants in the third series on which insects from the yellowed plantain had fed, became diseased. The aster plants on which the insects from the healthy plantain fed, and the plants on which one of the colonies from the diseased plantain fed, remained healthy. The plants were then fumigated, and approximately 100 virus-free nymphs were placed on one of the aster plants to which yellows had been transmitted from plantain. An equal number of virus-free nymphs were placed on one of the healthy aster plants. They were kept on these plants for 4 days. The 2 colonies were then transferred to 2 healthy plantain plants. They were left on these plants for 3 weeks. At the end of this period all insects were removed and the plants were fumigated. The plant on which insects from the yellowed aster had fed became diseased; that on which the insects from the healthy aster had fed remained healthy. The diseased plantain showed clearing of veins and other symptoms similar to those observed on the plantain found growing on the golf links. The experiment proves that the disease observed on

*Plantago major* early in the spring is aster yellows and that from such a plant it can be transmitted to asters by *Cicadula sexnotata*.

Ten virus-free adult leafhoppers were placed on yellowed specimens of each of the following plants transplanted from the golf links: *Chrysanthemum leucanthemum* L., *Sonchus arvensis* L., *Poa annua* L., and *Erigeron* sp. The same number of insects were placed on healthy specimens of each of these plants. After feeding on the plants for 6 days all insects were transferred to healthy aster plants on which they were allowed to feed for 3 weeks. The insects from the yellowed *Chrysanthemum*, the yellowed *Sonchus*, and the yellowed *Erigeron* transmitted yellows to the aster plants on which they fed. The insects from the yellowed *Poa annua* plant and those from the 4 check plants failed to transmit the disease. The experiment proves that the yellows found early in the spring on *Chrysanthemum leucanthemum*, *Sonchus arvensis*, and *Erigeron* sp. is aster yellows and that it can be transmitted from these plants to asters by *Cicadula*. It also shows that the yellowed *Poa annua* plants did not have aster yellows. Subsequent experiments in which attempts were made to transfer aster yellows from diseased asters to healthy-appearing *Poa annua* plants proved that this species is not susceptible to the aster disease. The yellowing of the *Poa annua* plants was probably due to some unfavorable soil condition, for when the yellowed plants were placed in rich garden soil they recovered and became normal green in color.

While the above-described experiments prove that the yellows appearing on several wild perennials in June is aster yellows, they do not definitely prove that the disease was carried over winter in these plants. In order to obtain direct evidence on this point, several *Chrysanthemum leucanthemum* plants and one *Plantago major* plant to which yellows was transmitted in the summer of 1925 were planted in a flower garden in the autumn. Healthy check plants of the same species were placed in the same garden. The diseased plants showed yellows on the new growth appearing in the spring of 1926. The experiment proves that these perennials carry the disease over winter. There are doubtless many other susceptible perennials on which the disease may pass the winter. It is thought, however, that the 3 species just mentioned are the most important winter carriers of the disease in the eastern United States.

The Paris daisy and other cultivated chrysanthemums grown in greenhouses also carry yellows over winter. Yellowed Paris daisies were found in several commercial greenhouses during the past two winters. Some of these plants were experimentally proven to have aster yellows. Cuttings made from such plants and put outside during the summer serve as foci of infection. A yellows reported by Nelson (18) on glasshouse-grown chrysanthemums in Michigan is probably identical with the aster yellows disease. *Cicadula sexnotata* is not a greenhouse insect, and so far as known aster yellows never spreads in commercial greenhouses. Many chrysanthemums

are propagated by cuttings which are often placed outside in garden plots during the summer. It is at this time that *Cicadula* transmits yellows to them, but the symptoms of the disease may not be noticeable when the plants are brought into greenhouses in the autumn.

#### INFLUENCE OF YELLOWS ON THE SUSCEPTIBILITY OF PLANTS TO OTHER DISEASES

A species of *Botrytis* which Prof. H. H. Whetzel has kindly informed me belongs to a *Botrytis* of the *cinerea* type is a common parasite of the China aster. It causes a damping-off of young plants and a wilt disease of old plants. It frequently forms small black sclerotia on aster seeds. The sclerotia carry the fungus to the seed bed, where numerous conidia are produced. This fungus attacks yellowed plants much more readily than healthy ones. It is the direct cause of the premature dying of most yellowed plants. The yellows disease apparently lowers the resistance of aster tissues to the fungus. It predisposes aster plants to attack by this *Botrytis* in much the same way that sugar cane mosaic predisposes sugar cane leaves to attack by the red-rot fungus *Colletotrichum falcatum* Went (13).

The leaf petioles of several yellowed and healthy aster plants were inoculated with the crown-gall organism, *Bacterium tumefaciens* Smith. Similar galls were produced on both yellowed and healthy plants. The galls on healthy leaves were green while those on yellowed leaves were chlorotic. No difference was observed between the susceptibility of healthy and that of yellowed tissues to this organism.

Aster yellows was readily transmitted to *Asclepias nivea* L., infected with the flagellate *Herpetomonas elmassiani* Migone, by means of *Cicadula sexnotata*. The flagellate flourished in the latex of the yellowed milkweed and seemed unaffected by yellows in spite of the fact that the plant itself showed marked symptoms. The latex of the yellowed plant is apparently as suitable a medium for the growth of the flagellate as is the latex of healthy plants. No noticeable change in the susceptibility of the plant to the flagellate was observed even when the yellows disease had reached an advanced stage.

#### INCUBATION PERIOD OF ASTER YELLOWS IN ITS INSECT CARRIER

During the winter of 1924 several experiments were undertaken to test the ability of the different instars of the nymphs of *Cicadula sexnotata* to transmit aster yellows. Cultures consisting of 100 nymphs in each of the 5 different instars were used. The nymphs were hatched and kept until the time when they were tested on yellowed aster plants. Twenty-five adult insects from similar cultures were used as a check. Each culture was caged separately on a healthy young aster plant for 2 days. The insects were then removed. The plants were fumigated and placed in an insect-proof cage. After 17 days the plant exposed to adult insects became diseased. The other

5 plants that were exposed to nymphs remained healthy during the 2 months that they were kept under observation. The experiment was repeated and again gave the same results.

It was thought that the nymphs might not feed as deeply in the plant tissues as do adults, and that they might be able to transmit the disease if very young plants were used. Nymphs in the fourth and fifth instars were accordingly placed on young aster plants having 2 pairs of leaves only. The tissues of such plants are very tender and easily pierced, but the nymphs failed to transmit the disease. Other experiments proved that adults from nymphs incapable of transmitting yellows can transmit the disease without feeding on yellowed plants after reaching maturity. The nymphs take up the virus. Further experiments showed that virus-free adult insects are unable to transmit yellows directly after feeding on diseased plants. A period of approximately 2 weeks must elapse between the time they feed on such plants and the time when they are first capable of transmitting the virus. This interval is referred to as the incubation period of the virus in the insect. The virus of curly-top of beets has been shown to go through a similar but much shorter incubation period in the beet leafhopper (27, 24). It was found that all instars of the nymphs of *Cicadula* are capable of taking up the virus. They are unable to transmit it because the incubation period in the insect is usually longer than the period necessary for the nymphs to reach maturity.

It was found, however, that under certain circumstances the incubation period is shorter than the time necessary for nymphs to reach maturity, and in that case they are able to transmit the disease. In one experiment 80 newly hatched nymphs were confined on a yellowed aster plant for 14 days. They were then confined for successive periods of time on 3 healthy young aster plants. They were kept on the first plant 4 days, on the second plant 4 days, and on the third plant 7 days. The first plant remained healthy, but the second and third plants became diseased. None of the insects had reached maturity at the end of the 22d day, when they were transferred from the second plant. The virus had, however, completed its incubation period, and the nymphs transmitted the disease. This result was obtained with a culture of insects kept at a relatively low temperature. At temperatures of 70° F. or above most of the insects mature before the incubation period is completed.

The fact that an incubation period is necessary before insects that obtain the virus become inoculative is considered to be important evidence as to the nature of the virus. For this reason the incubation period was carefully studied. Thirty-four experiments were performed which show the existence and approximate length of the incubation period in insects of different ages and with virus obtained from different host plants. The details of some of these experiments are shown in table 3. The numbers at the head of each column in the table indicate the dates on which plants were

exposed to virus-carrying insects. The insects remained on each plant until the next date shown in the table. Plants that remained healthy are indicated by the plus sign; those that became diseased, by the double plus sign. All plants used in the experiments summarized in the table were kept under daily observation. The numbers immediately below each double plus sign show the number of days between the time the plants were exposed to insects and the time when the first symptoms of disease were observed. These figures show in each case the incubation period of the disease in the plant. The first column in the table describes by number the cultures of insects used in each of the 7 experiments. The last column in the table gives, for each experiment, the length of the incubation period of the virus in the insects used. Vigorous colonies of insects were used in every case. Before some of the experiments were ended, however, the colonies had grown old. All plants were kept under observation until May 15.

The insect culture described as number 1 in the table consisted of 30 virus-free nymphs in the first, second, and third instars. The number in each instar was not recorded. The colony was placed on a yellowed *Calendula* plant on January 20. On January 25 it was removed and placed on the first plant shown in the table. The insects remained on this plant until January 28. From this date until March 13 they were transferred daily to new plants. They were then transferred to new plants every 2 days or after longer intervals as shown in the table. The culture was transferred at frequent intervals until April 15, when the experiment was ended, but only the results obtained to March 22 are shown in the table. The 10 plants on which the insects fed from January 25 to February 5 remained healthy. Of the 47 plants on which they fed from February 6 to April 15 all but 3 took the disease. The 3 plants that escaped infection were exposed after March 28, when the insect culture was becoming old and weak. Only 2 of the original 30 insects were alive on this date. The experiment shows an incubation period in the insect of from 12 to 17 days: 17 days if the virus was picked up on the first day the insects were on the yellowed *Calendula* plant, and 12 days if it was picked up on the last day.

The insect culture described in the table as number 2 consisted of 30 nymphs in the first instar when the experiment was started on January 15. The insects were hatched and kept on a yellowed aster plant until taken for use in the experiment. They varied in age from a few hours to 3 days. None was more than 3 days old. They were kept from January 15 to January 28 on 4 healthy young aster plants. The plants were exposed for various periods of time as shown in the table. All remained healthy. From January 28 to March 13 they were transferred daily to new plants. All plants exposed to the culture from January 29 to March 3 became diseased. All plants exposed after March 3 remained healthy. Twelve of the original 30 insects were living on this date. It is possible that these 12 insects were the youngest in the culture and that they were taken from the

TABLE 3. Incubation Period of the Virus of Aster Yellow in Plant and Insect Hosts

	January															February															Incubation in Insects												
	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	12-17 days	14-17 days	12 days	14-17 days	13-15 days	10-12 days	14-19 days		
Insect culture no. 1...																																											
Incubation in plant...																																											
Insect culture no. 2...	+																																										
Incubation in plant...																																											
Insect culture no. 3...																																											
Incubation in plant...																																											
Insect culture no. 4...																																											
Incubation in plant...																																											
Insect culture no. 5...	+																																										
Incubation in plant...																																											
Insect culture no. 6...																																											
Incubation in plant...																																											
Insect culture no. 7...																																											
Incubation in plant...																																											

	February															March															Incubation in Insects												
	18	19	20	21	22	23	24	25	26	27	28	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	12-17 days	14-17 days	12 days	14-17 days	13-15 days	10-12 days	14-19 days		
Insect culture no. 1...																																											
Incubation in plant...																																											
Insect culture no. 2...																																											
Incubation in plant...																																											
Insect culture no. 3...																																											
Incubation in plant...																																											
Insect culture no. 4...																																											
Incubation in plant...																																											

The table shows the incubation period of the virus in different insect colonies and the incubation period of the disease in different plants. The insect cultures are described by numbers from 1 to 7. The numbers at the top of each column show the dates during which plants were exposed. A plus sign (+) indicates a healthy plant and a double plus sign (++) a yellowed plant. The incubation periods of the virus in different insect colonies are shown in the last column.



diseased plant on which they hatched before they had opportunity to feed. An incubation period of the virus in the insects of from 14 to 17 days is shown by this experiment.

The insect culture described as number 3 in the table consisted of 100 virus-free adults when the experiment was started January 28. The insects were placed on a yellowed aster plant on January 28 and were removed on January 29. The first 36 plants used in this experiment are shown in Plate XL. The insect colony was kept on each plant for one day. The plant shown on the left side of the picture was diseased long before the experiment was started, and is the one from which the insects obtained the virus. They were kept on the plant exactly 24 hours. From January 29 to March 13 they were transferred daily to new plants. The 11 plants on which the insects fed from January 29 to February 8 remained healthy. The 21 plants on which they were cultured from February 9 to March 1 became diseased. The next 2 plants remained healthy, while the last of the 36 plants shown in the picture became diseased. The plants exposed on March 4, 7, 8, and 9 became diseased. The other plants exposed after March 1 remained healthy. Thirteen of the original 100 insects were living on that date. It is possible that most of these insects did not obtain the virus during the 24 hours they were on the diseased plant. The experiment shows an incubation period of 12 days.

The insect culture described as number 4 in the table consisted of 100 virus-free adults when the experiment was started. They were allowed to feed on a yellowed aster plant from January 26 to January 29. After this date they were transferred daily to new healthy young aster plants until March 7. All the 14 plants exposed from January 29 to February 11 remained healthy. All the 24 plants exposed between February 12 and March 7 became diseased except that exposed March 5, when the culture was old and weak. On that date, 16 of the original 100 insects were living. It is possible that these insects failed to obtain the virus during the 3 days they were on the yellowed aster plant. An incubation period in the insects of from 14 to 17 days is shown in this experiment; 17 days if the virus was picked up on the first day the insects were on the yellowed plant, and 14 days if it was picked up on the last day.

The insect culture described as number 5 in the table consisted of 30 virus-free nymphs in the first instar when the experiment was started. They were confined on a yellowed *Calendula* plant from January 13 to January 15. They were then transferred to new plants at intervals, as shown in the table. From January 28 to February 17 they were transferred daily. The 3 plants on which the insects fed from January 15 to January 27 remained healthy. The 21 plants on which they fed from January 28 to February 17 became diseased. An incubation period in the insects of from 13 to 15 days is shown in this experiment; 15 days, if the virus was picked up on the first day the insects were on the yellowed *Calendula* plant, and 13 days if it was picked up on the last day.



The insect culture described as number 6 in the table consisted of 30 virus-free adults when the experiment was started. They were confined on a yellowed aster plant for 2 days, from January 26 to January 28. From January 28 to February 17 they were transferred daily to new healthy young aster plants. All the plants on which they fed from January 28 to February 6 remained healthy. All the plants on which they fed after February 6 became diseased. An incubation period of from 10 to 12 days is shown in this experiment; 12 days if the virus was picked up on the first day the insects were on the yellowed aster plant, and 10 days if it was picked up on the last day.

The insect culture described as number 7 in the table consisted of 30 nymphs in the first, second, and third instars when the experiment was started. No record was made of the numbers in each instar. The insects were placed on a yellowed *Calendula* plant on January 20 and were kept on the plant until January 25. On the latter date they were placed on a healthy young aster plant. They were kept on this plant for 3 days, after which they were transferred daily until February 17. The twelve plants on which they fed from January 25 to February 7 remained healthy. The 10 plants on which they fed from February 8 to February 17 became diseased. The experiment shows an incubation period in the insect of from 14 to 19 days.

The results obtained with the different insect colonies described in the table are in close agreement, although some variation is shown in the length of the incubation period in the different colonies. All the experiments show that virus-free insects are unable to transmit yellows immediately after feeding on yellowed plants. An interval of at least 10 days was necessary in every case before any of the colonies were inoculative. With one exception, 10 days is the minimum incubation period shown by the virus in the 34 insect colonies that have been tested. In one experiment a plant, exposed 6 days after the insects used had first fed on a yellowed plant, became diseased. Six other plants following this one in the series, and exposed to the insects after this plant, remained healthy. It is thought that the plant had been accidentally inoculated before being used in the experiment. If this assumption is correct, the minimum incubation period is not less than 10 days. It is interesting to note that the minimum incubation period of the virus in the insect is of approximately the same length as the minimum incubation period of the disease in aster plants.

A study of the table will show that the incubation period of the disease in different aster plants varies between rather wide limits. The average incubation period in the plants used in the experiments summarized in the table is about 18 days. The shortest period shown is by a plant used in experiment number 1 and exposed February 19. The longest periods shown are by plants exposed on February 6 and February 12 in experiments numbers 1 and 4. The plant in experiment number 1 gave an incubation period of 39 days; that in experiment number 4, one of 38 days. This

period is considerably longer than that shown by any of the other plants and is more than twice the average incubation period shown by the other plants. It is interesting that the disease was transmitted to both these plants by insects in which the virus had just finished its incubation period. This suggests that the changes which probably occur during the incubation period of the virus in the insects may not have been completed when the virus was transmitted to the plants, but that they had progressed sufficiently to make possible a slow development of the disease in the plants. That the condition of the virus in the insect can influence the length of the incubation period in the plant is evidence in favor of the view that the causative agent is biological rather than chemical.

#### RETENTION OF THE VIRUS BY INSECTS CULTURED ON IMMUNE PLANTS

Many attempts were made to determine whether colonies of insects confined on plants immune to yellows would lose the virus.

On December 4, 1925, 30 nymphs in the first instar, which had been hatched and kept on a yellowed plant, were transferred to rye plants. They were kept on these plants for 33 days. The rye plants remained healthy in appearance. The 25 insects that were alive at the end of this period were transferred to a healthy young aster plant. After 6 days they were removed and placed on a second aster plant. After 11 days they were placed on a third, and after 7 days on a fourth, aster plant. They were kept on this last plant for one week. All the aster plants became diseased. The rye plants on which the cultures had been kept for 33 days were fumigated and placed in another cage. Thirty virus-free nymphs were confined on these plants for one month. They were then transferred to a succession of 4 healthy aster plants. They were kept on each of these plants for one week. The four plants remained healthy during the 2 months they were kept under observation. The experiment proves that nymphs in the first instar take up the virus and are able to retain it for at least 33 days when kept on rye plants. It also shows that rye plants do not carry the virus.

On November 18, 1925, 30 nymphs in the first instar, hatched and kept on a yellowed aster plant, were transferred to rye plants. They were kept on these plants for a period of 7 weeks. The colony was then transferred successively to 4 different aster plants. It was kept on the first plant 6 days, on the second, 11 days, on the third, 7 days, and on the fourth, 8 days. All the aster plants became diseased. The rye plants were fumigated and placed in an insect-proof cage. Thirty virus-free nymphs in different instars were confined on these plants for 2 weeks. They were then transferred successively to 4 healthy aster plants. They were kept on these plants for periods of 6, 11, 7, and 8 days. All 4 plants remained healthy during 2 months that they were kept under observation. The experiment proves that the virus is retained by the insects for at least 49 days.

On January 19, 1926, 100 nymphs in the first instar, hatched and kept on a yellowed aster plant, were transferred to rye plants. The colony was kept on these plants for 2 months. On March 19, the insects were taken from the rye plants and transferred successively at intervals of a few days to 8 different healthy young aster plants. The transfers were made on the following dates: March 19, 22, 25, 27, 29, 31, April 1, and April 5. All 8 aster plants exposed to these insects became diseased. The rye plants were fumigated, and 30 virus-free nymphs were confined on them for 2 weeks. The colony was then removed and transferred successively to 4 different aster plants. The insects were confined on each of these plants for 7 days. All remained healthy. The experiment proves that the virus was retained by the insects during the 59 days they were on the rye plants and during an additional 16 days that they were kept on healthy aster plants. Only 7 of the original 100 insects were alive when the experiment was ended. They retained the virus for a period of 75 days. During this time the insects passed through the 5 instars to maturity and old age. The fact that they retain the virus over such a long period of time is considered to be evidence in favor of the view that the causative agent is biological rather than chemical.

#### INSECT EXPOSURE TO YELLOWED ASTER PLANTS

It was observed in early experiments that certainty of transmission of aster yellows by cultures of *Cicadula sexnotata* depends on the number of insects used and on the length of time they are kept on yellowed plants. Many insect cultures readily transmit yellows when young but fail to transmit it when they grow old. Old colonies of insects are always reduced in numbers. It was thought that virus-carrying individuals might be shorter-lived than virus-free individuals, and that, when cultures grow old, only virus-free insects are left.

Several experiments were made to determine the exposure necessary for a medium-sized insect culture to obtain the yellows virus. Twenty virus-free adults were confined for 2 hours on each of 3 yellowed aster plants. Twenty similar adults confined for the same period of time on a healthy plant served as a check culture. Each culture was then transferred to a succession of 4 healthy aster plants. The colonies were kept on each plant for one week. All plants remained healthy, showing that a feeding period of 2 hours is not long enough to render colonies virus-bearing.

In another experiment, 25 virus-free adults were confined on a yellowed aster plant for one day. Twenty-five similar adults kept for the same length of time on a healthy aster plant served as a check culture. Both cultures were transferred to a succession of 4 healthy aster plants. They were confined on each plant for one week. The last 2 plants on which the insects exposed to the diseased aster fed became diseased. All other plants remained healthy. The experiment shows that an exposure of one day is

sufficient to enable the insects to become virus-bearing. The number of insects that obtain the virus in that time is not shown by this experiment.

It was suggested that insects might be able to take the virus from young leaves only, and that those insects that happened to feed on old leaves during their exposure to yellowed plants might not become disease carriers. An experiment was undertaken to test this hypothesis. Fifty virus-free nymphs in the first instar were placed in a large test tube containing old aster leaves from a yellowed plant. Thirty virus-free adults were placed on similar leaves in another tube. Fifty virus-free nymphs and 30 virus-free adults were placed in 2 other tubes containing young leaves from yellowed aster plants. Fifty similar nymphs and 30 virus-free adults placed in 2 other tubes containing both old and young leaves from healthy aster plants served as check cultures. All insects were kept in the tubes for 2 days. Each culture was then transferred to a succession of 4 healthy young aster plants. The insects were kept on the first set of plants for 10 days; on the second set for 5 days; on the third set for 6 days; and on the fourth set for 2 days. The last 2 plants on which the insect cultures exposed to diseased leaves fed became diseased in every case. All other plants remained healthy. The experiment proves that both nymphs and adults are able to obtain the virus from both old and young leaves.

It was also thought possible that certain individual insects might be immune to the virus and might be able to feed on yellowed plants indefinitely without becoming carriers of the disease. An experiment was, therefore, undertaken in which individual insects were employed for yellows-transmission. All the insects used in this experiment were from eggs laid in yellowed aster plants, February 19, 1926. The plants were kept in a greenhouse held at temperatures between 70° and 75° F. By March 24 many of the insects hatched from these eggs were adults. Since, at the temperatures at which the eggs were held, it takes about 12 days for them to hatch, it may be assumed that most of the insects were about 3 weeks old on this date. One of these young adults was placed in each of 30 lantern-globe cages. Thirty similar adults were placed in a single cage. The colony of 30 insects was designated as culture A. The other 30 insects were given culture numbers from 1 to 30. A succession of 23 healthy young aster plants were exposed to the colony and to each of the individual insects that lived until the experiment was ended. The different sets of plants were exposed for varying periods of time. None was exposed for less than one day and none for more than 7 days. In this way the transmission record of the colony and of each of the 30 individual insects has been obtained. The exposures were made on the dates and for the periods of time shown in table 4. All plants were kept under observation for at least 6 weeks after the insects were removed. The results are shown in the table. The plus sign indicates that the plant remained healthy; the double plus sign that it became diseased; and the asterisk that it died before the incubation period

TABLE 4. *Individual Transmission Records*

Culture number . . . . .	A	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Exposed March 24-March 30. . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
March 30-April 5. . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
April 5-April 10. . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
April 10-April 16. . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
April 16-April 21. . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
April 21-April 28. . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
April 28-April 29. . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
April 29-April 30. . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
April 30-May 1. . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
May 1-May 3. . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
May 3-May 4. . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
May 4-May 5. . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
May 5-May 7. . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
May 7-May 10. . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
May 10-May 14. . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
May 14-May 21. . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
May 21-May 28. . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
May 28-June 4. . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
June 4-June 11. . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
June 11-June 18. . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
June 18-June 25. . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
June 25-July 2. . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
July 2-July 9. . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Transmission records of 30 individual insects and of a colony consisting of 30 insects. A plus sign (+) indicates a healthy plant; a double plus sign (++) a yellowed plant; an asterisk (\*) a dead plant. A minus sign (-) indicates the death of the insect used. The dates given in the first column show the periods during which each set of plants was exposed.

for the disease in the plant was ended. The minus sign indicates that the insect was dead on the date of beginning the exposure under which the sign appears.

It will be seen that the incubation period of the virus in most of the insects had not been completed on March 30, when the first exposure was ended. Only the colony and insects numbers 4 and 6 transmitted the disease. During the exposure of the second set of plants the colony and insects numbers 1, 2, 4, 6, 8, 11, 13, 14, 20, 24, and 30 transmitted the disease. During the exposure of the third set of plants the colony and all the insects except numbers 2, 3, 9, 15, 16, 18, 19, 21, 23, 26, 27, and 28 transmitted yellows. Insect number 26 died during the exposure of the third plant. During the exposure of the fourth set of plants all the insects except numbers 3 and 28 transmitted the virus. In the fifth exposure all transmitted it except number 3. Numbers 3, 17, and 25 died during this exposure. The sixth set of plants was exposed for 7 days. The plant on which insect number 24 fed died prematurely. The disease was transmitted to all other plants except the 2 on which insects numbers 29 and 30 were confined. Insect number 14 died during the exposure. The seventh set of plants was exposed for only one day. During this period all insects transmitted the virus except numbers 5, 6, 18, 27, and 30. Insect number 27 died during this exposure. The eighth set of plants was also exposed for only one day. All insects transmitted the virus except numbers 5, 6, 18, 22, 23, and 28. The ninth set of plants was exposed for one day. All insects transmitted the virus except numbers 5, 6, 11, 18, 22, 23, 29, and 30. The tenth set of plants was exposed for two days. The virus was transmitted by all insects except numbers 5, 6, 18, 19, 22, 23, and 30. Insect number 2 died during the exposure. The eleventh set of plants was exposed for one day. The disease was transmitted by all insects except numbers 4, 5, 6, 7, 8, 10, 18, 22, 23, 28, and 29. The twelfth set of plants was exposed for one day, and the virus was transmitted by all insects except numbers 5, 6, 7, 10, 18, 19, 22, 23, 28, and 29. The plant exposed to insect number 19 died prematurely. The thirteenth set of plants was exposed for two days. All insects transmitted the virus except numbers 5, 6, 7, 8, 18, 22, 28, and 30. The plants on which numbers 5 and 6 fed died prematurely. Insect number 30 died during the exposure. The fourteenth set of plants was exposed for 3 days. The plant on which insect number 5 fed died before the transmission record was obtained. The virus was transmitted by all other insects except numbers 6, 8, 18, and 22. The fifteenth set of plants was exposed for 4 days. The plant on which insect number 5 was confined died prematurely. The disease was transmitted to the plants on which all other insects fed except numbers 6, 7, 8, 10, 18, 22, 23, 24, and 28. Insect number 6 died during this exposure. The sixteenth set of plants was exposed for 7 days. The plants on which insects numbers 18 and 20 fed died prematurely. All insects transmitted the disease except numbers 5, 8, 18, 20, and 22. Insects numbers

1 and 20 died during the exposure. The seventeenth set of plants was exposed for 7 days. All insects transmitted the virus except numbers 18, 19, and 22. Insect number 19 died during the exposure. The eighteenth set of plants was exposed for 7 days. The plant on which insect number 22 fed died before the transmission record was obtained. All other insects transmitted the virus except numbers 10, 11, and 18. The nineteenth set of plants was exposed for 7 days. All insects transmitted the virus except numbers 7, 10, 11, 18, 22 and 23. The twentieth set of plants was exposed for 7 days. All insects transmitted the disease except numbers 10, 18, 22, and 24. The twenty-first set of plants was exposed for 7 days. The plant on which insect number 12 fed died prematurely. Insects numbers 7 and 10 died during the exposure. All other insects transmitted the virus except numbers 7, 8, 10, 18, 22, and 23. The twenty-second set of plants was exposed for 7 days. All insects transmitted the disease except numbers 12, 18, 22, and 23. The one insect that remained alive in culture *A* failed to transmit the virus. It died during this exposure. Insect number 29 also died. The twenty-third set of plants was exposed for 7 days. All insects transmitted the virus except numbers 18, 22, and 23. On July 9, when the experiment was ended, only 6 of the insects were living.

The table shows the transmission record of culture *A* and of the several individual insect cultures used. Yellows was transmitted to all plants exposed to culture *A* except the last. The single insect that was living when this plant was exposed died during the exposure. The record shows that the culture was inoculative for at least 80 days, from March 30 to June 18. Yellows was transmitted to all except the first plant exposed to insect number 1. During the time of this exposure the virus had doubtless not completed its incubation period in the insect. This leafhopper died during the exposure of the sixteenth plant. It, nevertheless, transmitted yellows to this plant and to all the other 15 plants on which it fed after the termination of the incubation period of the virus. The insect was inoculative for at least 39 days, from April 5 to May 14. Insect number 2 was not inoculative during the time the first plant was exposed. It transmitted yellows to the plant exposed from March 30 to April 5, but failed to transmit it to the plant on which it fed from April 5 to April 10. The virus was carried to all other plants on which it was confined. The insect died during the exposure of the tenth plant, but transmitted yellows to this plant. It was inoculative during a period of at least 26 days, from April 5 to May 1. Why it did not transmit yellows to the plant on which it fed from April 5 to April 10 is not known. Insect number 3 died during the exposure of the fifth set of plants. It failed to transmit the disease to any of the plants on which it fed. This does not prove that it did not obtain the virus. The insect died during the exposure of the fifth plant, and may not have fed on this plant. Insect number 28 also failed to transmit yellows to the first 4 plants on which it was confined. It is assumed that the incubation period of the virus in insects



numbers 3 and 28 was not completed during the exposure of the first 4 sets of plants. Insect number 4 transmitted yellows to all but one of the 20 plants on which it was confined. This plant was exposed for only one day. It was inoculative for a period of at least 73 days, from March 30 to June 11. Insect number 5 failed to transmit yellows to the first 2 plants on which it fed. The incubation period of the virus was probably not completed during the time these plants were exposed. It transmitted the disease to the next 4 plants, but failed to transmit it to the 10 plants on which it fed from April 28 to May 21. Three of these plants died prematurely. It was apparently unable to transmit yellows to plants on which it was confined for no longer than one or two days, and even failed to transmit it to plant number 16 on which it was confined for one week. It did, however, transmit the virus to all the 4 plants on which it fed during the last 4 weeks of its life. Insect number 6 transmitted the virus to the first 6 plants on which it was confined. It failed to transmit it to any of the plants exposed after April 28. Insect number 7 failed to transmit yellows to the first 2 plants, transmitted it to the next 8 plants, failed to transmit it to the next 3, transmitted it to the fourteenth plant, failed to transmit it to the fifteenth plant, transmitted it to the sixteenth, seventeenth, and eighteenth plants, failed to transmit it to the nineteenth plant, transmitted it to the twentieth plant, and failed to transmit it to the twenty-first plant. This insect was inoculative over a period of at least 62 days, but for some reason did not transmit the disease to many of the plants on which it was confined. Insect number 8 also transmitted yellows over a long period of time, but without taking it to all plants exposed. It was inoculative for at least 88 days, from April 5 to July 2. After the completion of the incubation period it carried the virus to all but 6 of the 22 plants on which it fed. Insect number 9 failed to transmit the virus to the first 3 plants on which it was confined. The incubation period had undoubtedly not been completed during the time these plants were exposed. After this period was terminated the insect transmitted yellows to all the 20 plants on which it was confined. It was inoculative for a period of at least 77 days. Insect number 10 failed to transmit yellows to the first 2 plants on which it was confined, but carried it to 12 of the 19 plants on which it fed during the remainder of its life. Insect number 11 failed to take yellows to the first plant but transmitted it to all but one of the next 16 plants. The 2 plants on which it was confined shortly before its death remained healthy. Insect number 12 failed to transmit yellows to the first 2 plants. It carried the disease to all but 2 of the other 21 plants. One of these plants died prematurely; the other remained healthy. This insect was capable of inoculating plants over a period of at least 83 days. Insect number 13 failed to transmit yellows to the first plant on which it was confined, but carried it to all the other 17 plants on which it fed until the time of its death. Insect number 14 carried yellows to all except the first plant. It died prematurely. Insect number 15 transmitted



the disease to all except the first 3 plants. It was inoculative over a period of at least 56 days. The transmission record of insect number 16 is exactly like that of insect number 15, except that number 15 lived a few days longer and transmitted yellows to one more plant than did number 16. Insect number 17 died prematurely. It failed to transmit yellows to the first 2 plants on which it was confined but carried it to 3 other plants. Insect number 18 failed to transmit yellows to the first 3 plants but took it to the next 3. It failed to transmit the disease to any of the plants on which it fed thereafter. Insect number 19 also failed to transmit yellows to the first 3 plants. It carried the disease to 11 of the other 14 plants on which it fed. One of the 3 healthy plants died prematurely. The other 2 remained healthy. Insect number 20 transmitted yellows to all the plants on which it was confined except the first and last. The last plant died before the transmission record was obtained. The insect was inoculative for at least 35 days. Insect number 21 transmitted the virus to all plants on which it was confined except the first 3. It was inoculative for at least 49 days. Insect number 22 failed to transmit yellows to the first 2 plants but carried it to all the next 5. It failed to transmit the disease to any of the 16 plants on which it fed thereafter. This insect was inoculative for not more than 19 days. Insect number 23 failed to transmit yellows to the first 3 plants but did transmit it to the next 4. It carried the disease to only 6 of the next 16 plants on which it was confined. Insect number 24 transmitted yellows to all plants on which it was confined except the first, sixth, fifteenth, and twentieth. No record was obtained for the sixth plant because of its early death. The insect died during the exposure of the twentieth plant. Insect number 25 died during the exposure of the fifth plant. It transmitted yellows to all except the first 2 plants. Insect number 26 died during the exposure of the third plant. It failed to transmit yellows to the plants on which it fed. The virus had probably not completed its incubation period when the insect died. Insect number 27 failed to transmit yellows to the first 3 plants, but carried it to the fourth, fifth, and sixth plants. It died during the exposure of the seventh plant, to which it failed to transmit the disease. Insect number 28 failed to transmit yellows to the first 4 plants but carried it to the next 3 and to all but 5 of the 11 plants on which it was confined during the remainder of its life. Insect number 29 failed to take yellows to the first 2 plants but carried it to all but 4 of the remaining 20 plants. Insect number 30 failed to transmit yellows to the first plant on which it fed, but did transmit it to all but 5 of the other 12 plants exposed.

These 30 transmission records show that all insects that lived long enough to give a transmission record were virus-carriers. Both males and females were included in the group of individuals tested. Insects numbers 4, 13, and 15 were females. Insects numbers 5 and 28 were males. Considerable variation is shown in the certainty with which different individuals

transmit the disease. Ten insects transmitted it to every plant on which they were confined after the completion of the incubation period. Three of these, numbers 14, 17, and 25, were short-lived. The other 7, consisting of numbers 1, 9, 13, 15, 16, 20, and 21, transmitted yellows over a long period of time and with great certainty. A few other insects, like numbers 2, 4, and 12, transmitted it to almost all the plants on which they fed and should probably be placed in this class. Another group of insects, of which numbers 5, 7, 8, 10, 23, 28, 29, and 30 are examples, transmitted yellows over a long period of time but with considerable irregularity. Many of the plants on which they were confined escaped the disease. Still another group transmitted yellows to a few plants and then apparently lost the virus. Insect number 6 transmitted the disease to the first 6 plants on which it fed but failed to transmit it to any of the 8 plants on which it was later confined. After the completion of the incubation period of the virus, insect number 18 transmitted yellows to 3 plants. It was kept on the first, second, and third plants for periods of 6, 5, and 7 days respectively. It failed to transmit yellows to any of the 16 plants on which it was confined during the following 72 days. Number 22 is another example of an insect that lost its ability to transmit yellows. It carried the disease to the first 5 plants on which it fed after the completion of the incubation period. It failed to transmit it to any of the 15 plants on which it was confined during the following 71 days.

It is interesting to note that 6 of the 30 insects lived during the 107 days this experiment was in progress. They were all from eggs deposited 33 days before the experiment was started. If the eggs hatched in 12 days, these insects were 128 days old when the experiment was ended. When *Cicadula sexnotata* is confined in large colonies, its average length of life is not more than 60 days. All but one of the insects in culture A died before May 28. Eighteen of the 30 insects kept in individual cages were alive on this date. *Cicadula* lives much longer in individual cages than it does when grown in colonies, even though the colonies are small and are given an abundant food supply. Three of the insects alive when the experiment was ended transmitted yellows to the last plants on which they were confined, showing that they carried the virus for more than 100 days. The other 3 insects were not inoculative during the last 3 weeks of the experiment.

This experiment brings evidence that most, if not all, individuals of *C. sexnotata* are capable of taking up the aster yellows virus. Some transmit the disease to all plants on which they feed for as long as one day, while others transmit it with much less certainty. Many individuals carry the virus as long as they live. Some, however, appear to lose it after a short time.

#### EFFECT OF YELLOWS ON ITS INSECT CARRIER

When it was found that a specific relationship exists between aster yellows and its insect carrier, an effort was made to determine whether yellows has any observable effect on its insect host. Cultures of virus-carrying and virus-free leafhoppers were kept under identical conditions

of light, temperature, and humidity on similar host plants in cages in a greenhouse. It was thought that the virus-carrying insects might be shorter-lived than those that are virus-free. It was found, however, that the average length of life of virus-bearing insects is approximately the same as that of virus-free insects. Since the virus causes chlorosis in green plant tissues and partial or complete loss of pigment in colored flowers, it was thought that it might affect in some way the color of the virus-carrying insects. Such insects in various stages of development were carefully compared with virus-free insects in corresponding stages of development. No differences in color could be observed between the two kinds of insects. They were also compared for differences in size and tested for resistance to heat and desiccation, but no differences whatever could be noted. Virus-free and virus-bearing nymphs and adults were fixed in Carnoy's fixative, imbedded in paraffin in the usual way, sectioned with a microtome, and stained with Flemming's triple stain. This method of fixing and staining gave good preparations, but no differences could be found in the morphology or structure of internal organs or in the reactions of cells in the several tissues to the stains. There are certainly no conspicuous differences between insects that bear the virus and those that do not.

#### DISCUSSION

Aster yellows is an infectious chlorosis of the China aster prevalent in North America but not known in other parts of the world. Its characteristics place it in the group of virus diseases, but it has not been shown to be due to a filterable virus. It is transmitted by a leafhopper which was probably imported into the United States 50 or more years ago. This insect is common in Europe and the Orient, where the aster and other host plants of the disease are extensively grown. Aster yellows, however, does not occur in European and Oriental countries apparently because the virus of this disease has not yet reached them.

The aster yellows disease is doubtless endemic in North America. It probably occurred unnoticed on some wild host plant long before the European leafhopper *Cicadula sexnotata* and the Oriental aster *Callistephus chinensis* were brought to the United States. It may have been and may still be transmitted by some insect endemic in the United States. The disease was probably of little importance, however, until after the importation of *Cicadula sexnotata*. This insect, being very active and living and breeding on many different species of plants, makes aster yellows a serious disease and its control an important problem for those who grow the China aster or other cultivated host plants of the disease.

The most promising means of control that can be suggested at present are eradication of weed hosts in the vicinity of aster plantings, destruction of all aster plants as soon as they are observed to show yellows, and spraying or dusting aster plots with nicotine or other suitable sprays or dusts. Asters grown in plots surrounded by cultivated fields are less subject to severe

infection than asters grown in the vicinity of pastures, meadows, waste lands, or other weedy places. Aster beds near buildings are somewhat less subject to disease than plantings in the open, because the aster leafhopper is a wild insect and avoids buildings.

Leafhoppers, because of their activity and rapid multiplication, are difficult to control. It is fortunate, therefore, that only a relatively small number of virus diseases of plants seem to be transmitted by them. It has been known for many years that *Eutettix tenellus* Baker transmits the curly-top disease of sugar beets (1), and that *Nephotettix apicalis* Motsch. spreads the mosaic disease of rice.<sup>2</sup> More recently it has been shown that *Peregrinus maidis* Ashm. transmits the mosaic disease of corn (11), that *Empoa ulmi* L. spreads leaf roll of potato (17), and that *Balclutha mbila* Naude carries the streak disease of sugar cane and corn (29, 30). *Cicadula sexnotata* as the carrier of aster yellows must be added to this list. It is interesting to note that all these leafhoppers except *Peregrinus maidis* belong in the subfamily Jassinae.

A correct understanding of the spread and severity of aster yellows depends largely on a knowledge of the life and habits of *Cicadula sexnotata*. The fortunes of leafhopper colonies vary with weather conditions, with the prevalence of leafhopper diseases, and with the abundance of predacious insect enemies. Under any given set of conditions the incidence and severity of aster yellows are closely connected with the likes and dislikes of this leafhopper. The China aster is very susceptible to aster yellows infection. It is also a favorite host plant of *Cicadula*. These two facts account for the prevalence of yellows in aster plantings. The African marigold, though quite susceptible to infection, is not liked by the leafhopper. This plant may be grown adjacent to badly yellowed aster plots during a whole season without acquiring the disease. It rarely becomes infected when grown in gardens. If, however, a few inoculative leafhoppers are confined in a cage containing only marigold plants, the disease is quickly transmitted. When grown in gardens the plants remain free or almost free of yellows, not because they are resistant to the disease but because *Cicadula* prefers other host plants.

It is thought that the intimate and specific relationship which has been shown to exist between aster yellows and its insect carrier is important evidence in favor of the view that the causative entity is biological rather than chemical. It is difficult to conceive that any agent other than a living organism would require an incubation period in the insect carrier or would be retained by the insect for long periods of time in the absence of susceptible host plants. That the condition of the virus in the insect can influence the length of the incubation period of the disease in the plant suggests the occurrence of developmental changes in the virus during its incubation period in the insect.

<sup>2</sup>Takami, N. Stunt disease of rice and *Nephotettix apicalis*. Jour. Agr. Soc. Japan 241: 22-30. 1901. Mosaic or stunt disease of rice was the first virus disease of plants shown to be transmitted by an insect.

It is interesting to note that the average length of the incubation period of aster yellows in *C. sexnotata* is, at similar temperatures, approximately equal to the length of the incubation period of the malaria organism (*Plasmodium vivax*) in the mosquito *Anopheles*. It is also of the same order as the lengths of the incubation periods shown by *Trypanosoma rhodesiense* in the tsetse fly and by the yellow-fever organism (*Leptospira icteroides*) in the common house mosquito. So far as is known, incubation periods shown by disease-producing organisms in insects are due to a development of these organisms in some tissue or organ of the insect host. It seems probable that the incubation period of aster yellows in *Cicadula sexnotata* is due to a development and multiplication of the causative agent in some tissue of the leafhopper.

#### SUMMARY

1. Aster yellows is a serious disease of the China aster, *Callistephus chinensis* Nees. It is easily distinguished from all other aster diseases. Its characteristics show that it belongs in the virus-disease group.

2. Yellows was transmitted by budding but not by other mechanical means. It is transmitted by the leafhopper *Cicadula sexnotata* Fall. Evidence is brought that it is not transmitted by several other aster insects.

3. By means of this leafhopper the disease has been transmitted to more than 50 different species in 23 different families of plants. In the same way it has been carried from many of these different species back to aster. No evidence has been found that the virus is attenuated by passage through different host plants.

4. Both nymphs and adults are unable to transmit the virus immediately after feeding on yellowed plants. A period of at least 10 days must elapse before they become inoculative. This interval is referred to as the incubation period of the virus in the insect. The incubation period is somewhat shorter in adults than in nymphs. Many individuals retain the virus as long as they live, but some seem to lose it after a short time.

5. Aster yellows is not transmitted through the eggs of the insect carrier or through the seeds of the aster. It is not transmitted from yellowed to healthy plants by contact or directly from a virus-bearing insect to a virus-free insect. Individual insects have been proven to carry the virus for more than 100 days. Small colonies of insects confined on rye plants that are immune to yellows have been shown to retain it for at least 2 months.

6. Aster yellows is identical with white-heart disease of lettuce, with a previously undescribed disease of buckwheat, and with several yellows diseases of cultivated garden plants. It is similar to but apparently distinct from peach yellows, strawberry yellows, curly-top of beets, and false blossom of the cranberry. It is not identical with the stunt disease of Dahlia.

7. The disease overwinters in biennial and perennial host plants, some

of the most common of which belong in the genera *Chrysanthemum*, *Sonchus*, *Asclepias*, *Erigeron*, and *Plantago*.

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## EXPLANATION OF PLATES

### PLATE XL

*Cicadula sexnotata*. All figures  $\times 14$

- FIG. A. A nymph in the first instar.  
 FIG. B. A nymph in the second instar.  
 FIG. C. A nymph in the fifth instar.  
 FIG. D. A full-grown adult.

### Aster yellows Disease

FIG. E. The first 36 plants used in determining the incubation period of the virus of aster yellows in insect colony number 3, referred to in table 3. Each plant was exposed for a period of one day. The plant shown in the upper left corner of the figure is the one from which the insects obtained the virus. The next 11 plants remained healthy, showing that the insects were not inoculative during the periods of their exposure. The thirteenth plant shown in the figure was the first to which yellows was transmitted. The insects transmitted yellows to all the 21 plants on which they fed during the 3 weeks following the termination of the incubation period. The picture shows that these 21 plants were badly stunted by the disease. The thirty-fourth and thirty-fifth plants remained healthy, while the thirty-sixth plant became diseased.

### PLATE XLI

#### Host Range of the Aster yellows Disease

FIG. A. A healthy and a diseased plant of *Chrysanthemum frutescens* L. The diseased plant is stunted and chlorotic.

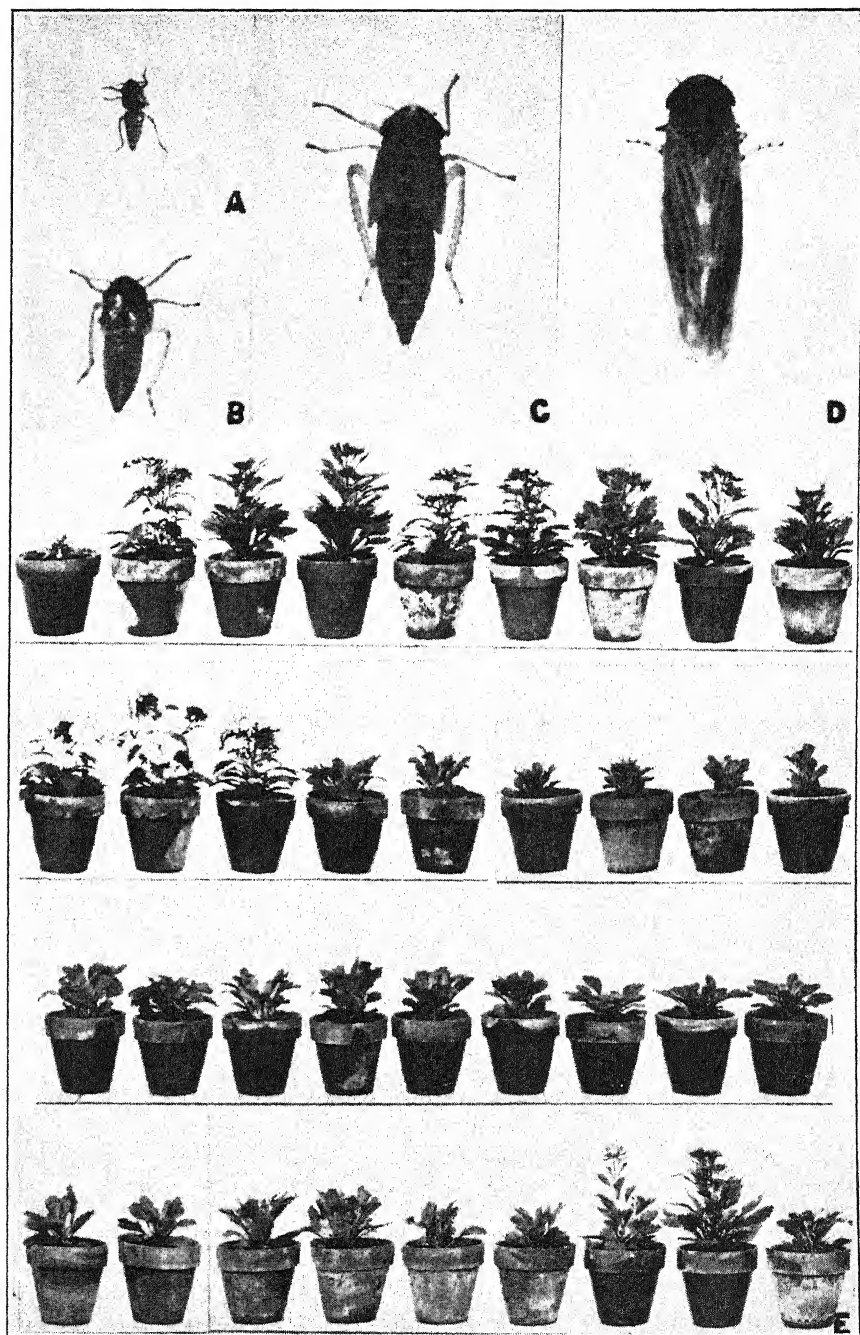
FIG. B. A healthy and a diseased plant of *Anethum graveolens* L. The diseased plant is dwarfed and chlorotic but has not produced secondary shoots.

FIG. C. A healthy and a diseased plant of *Matricaria alba*. The diseased plant is badly stunted and chlorotic. It has produced many secondary shoots.

FIG. D. A healthy and a diseased plant of *Dimorphotheca aurantiacum* DC. The diseased plant has produced many secondary shoots. It is dwarfed and chlorotic. The petals of the ray flowers are dwarfed and green in color.

FIG. E. A healthy and a diseased plant of *Centaurea margaritae* Hort. The diseased plant is chlorotic and has produced many upright secondary shoots. Some of the diseased leaves are twisted, have irregular margins, and are much longer than normal leaves of the same age.

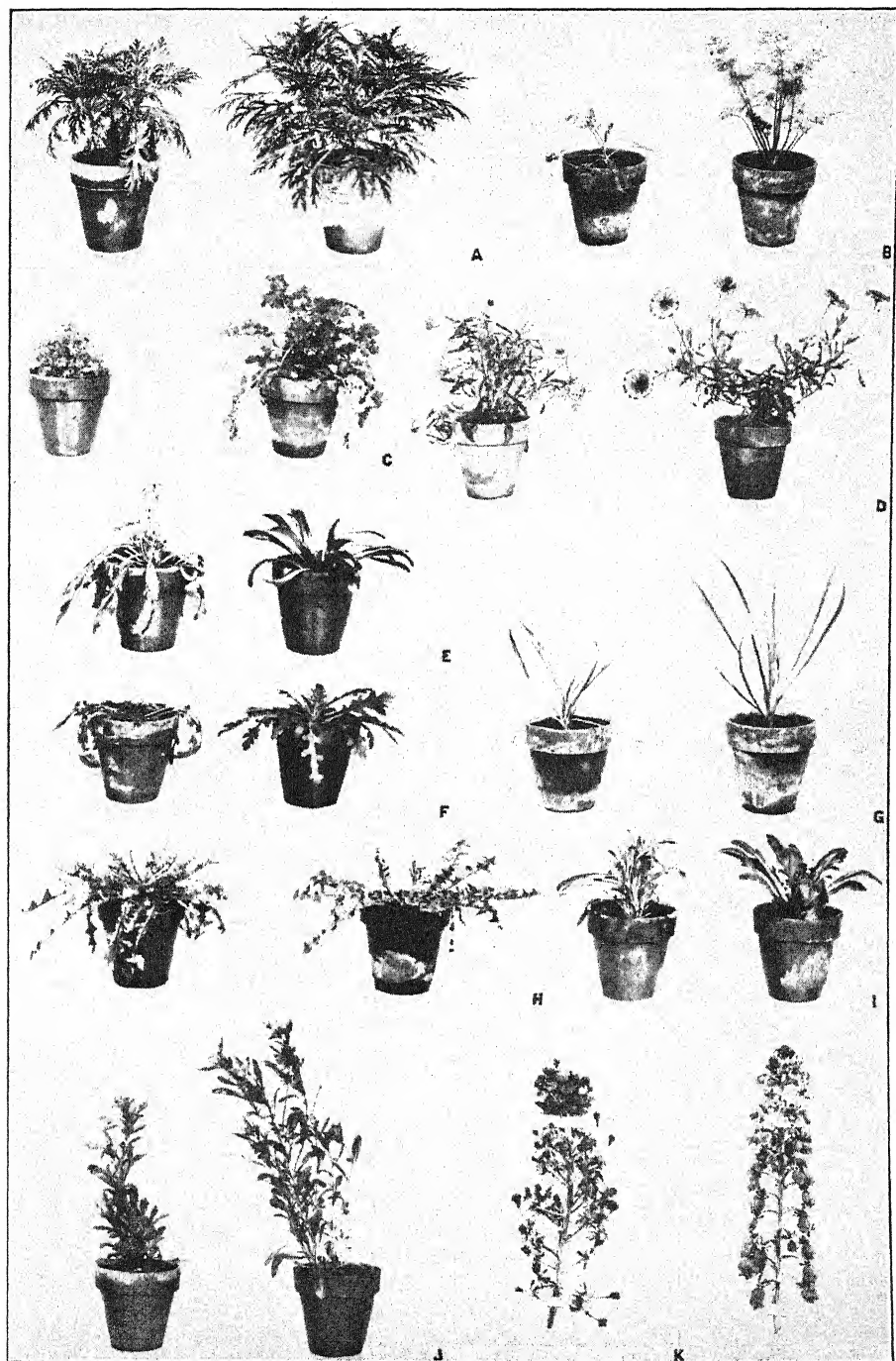




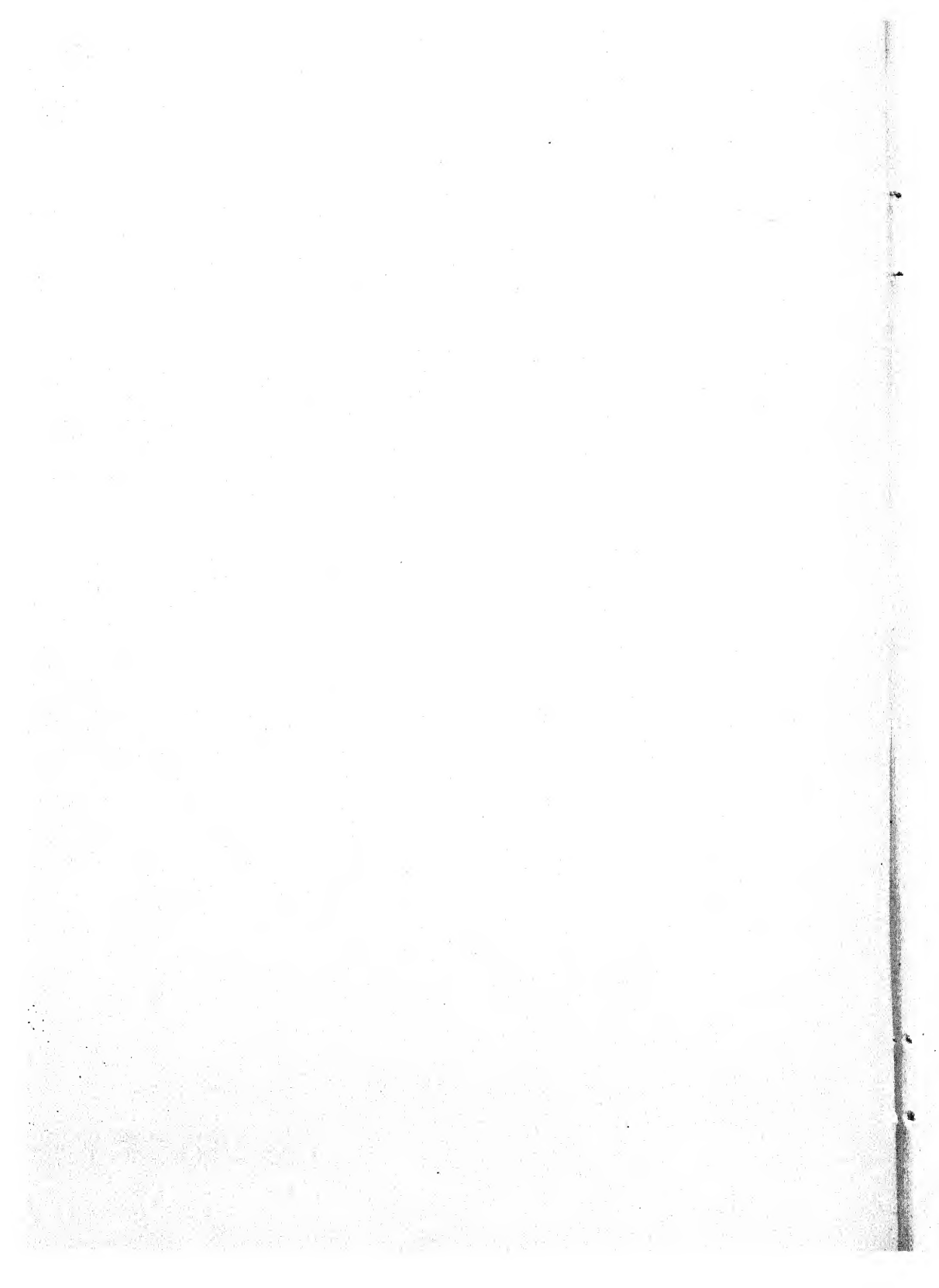
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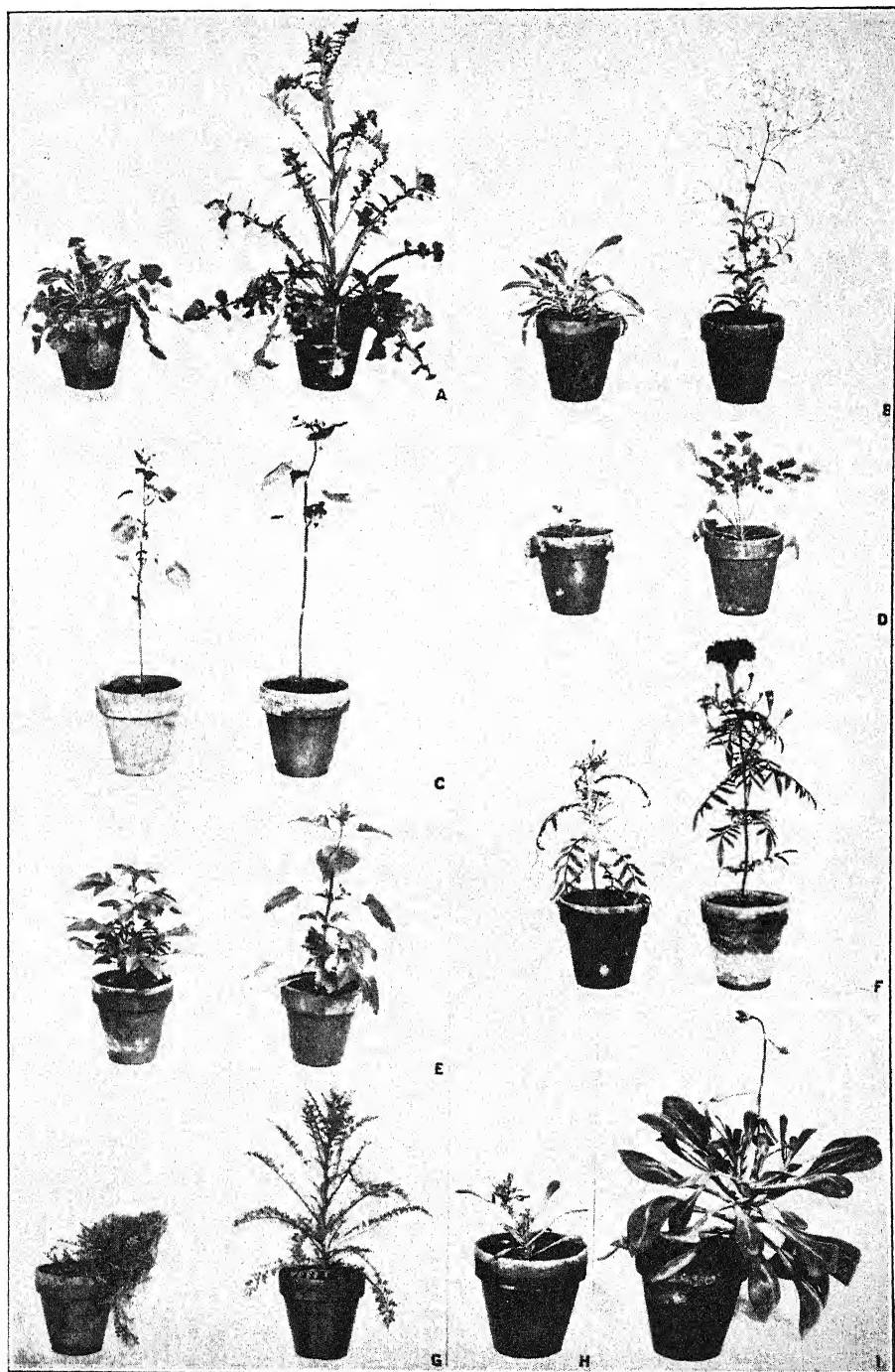




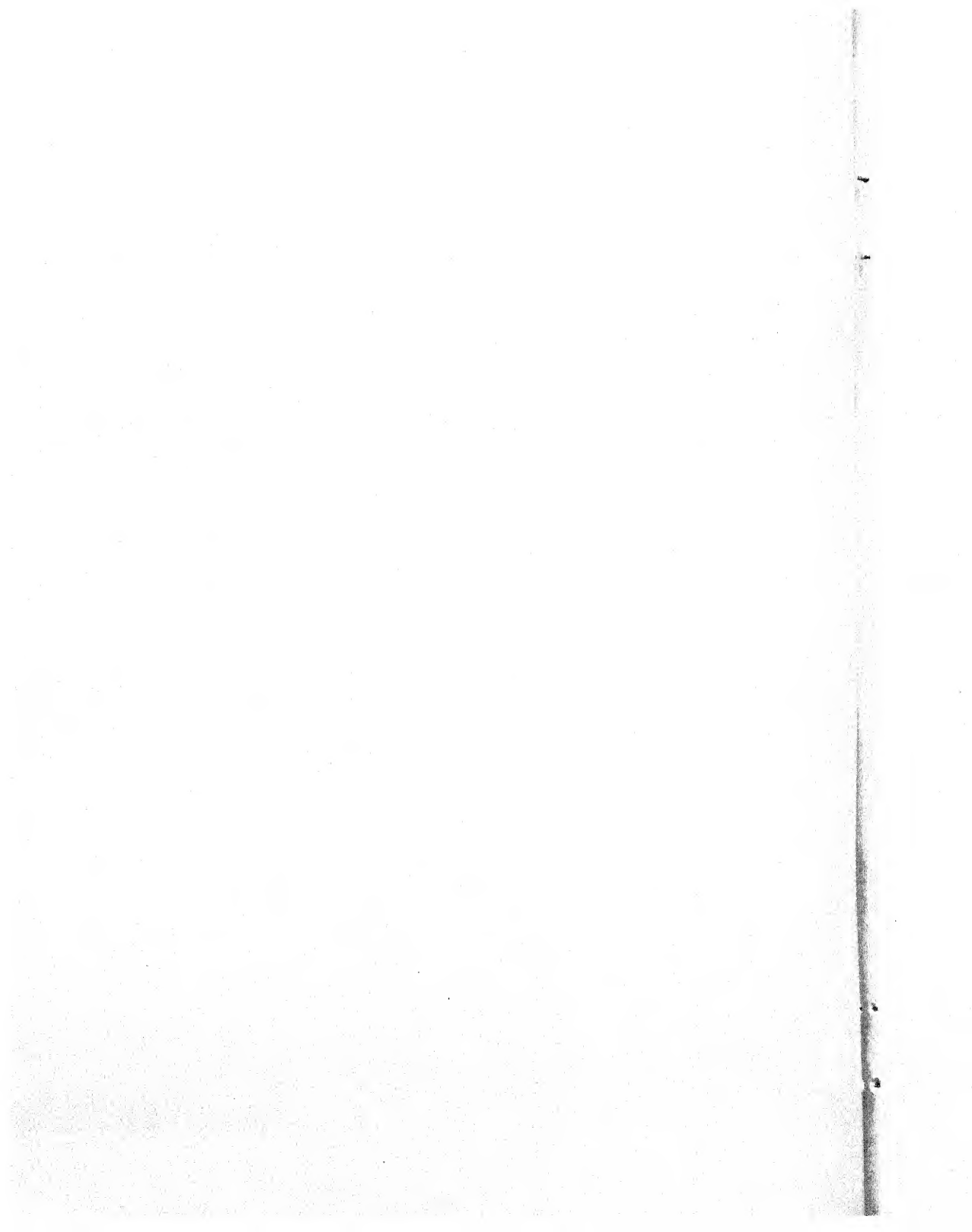


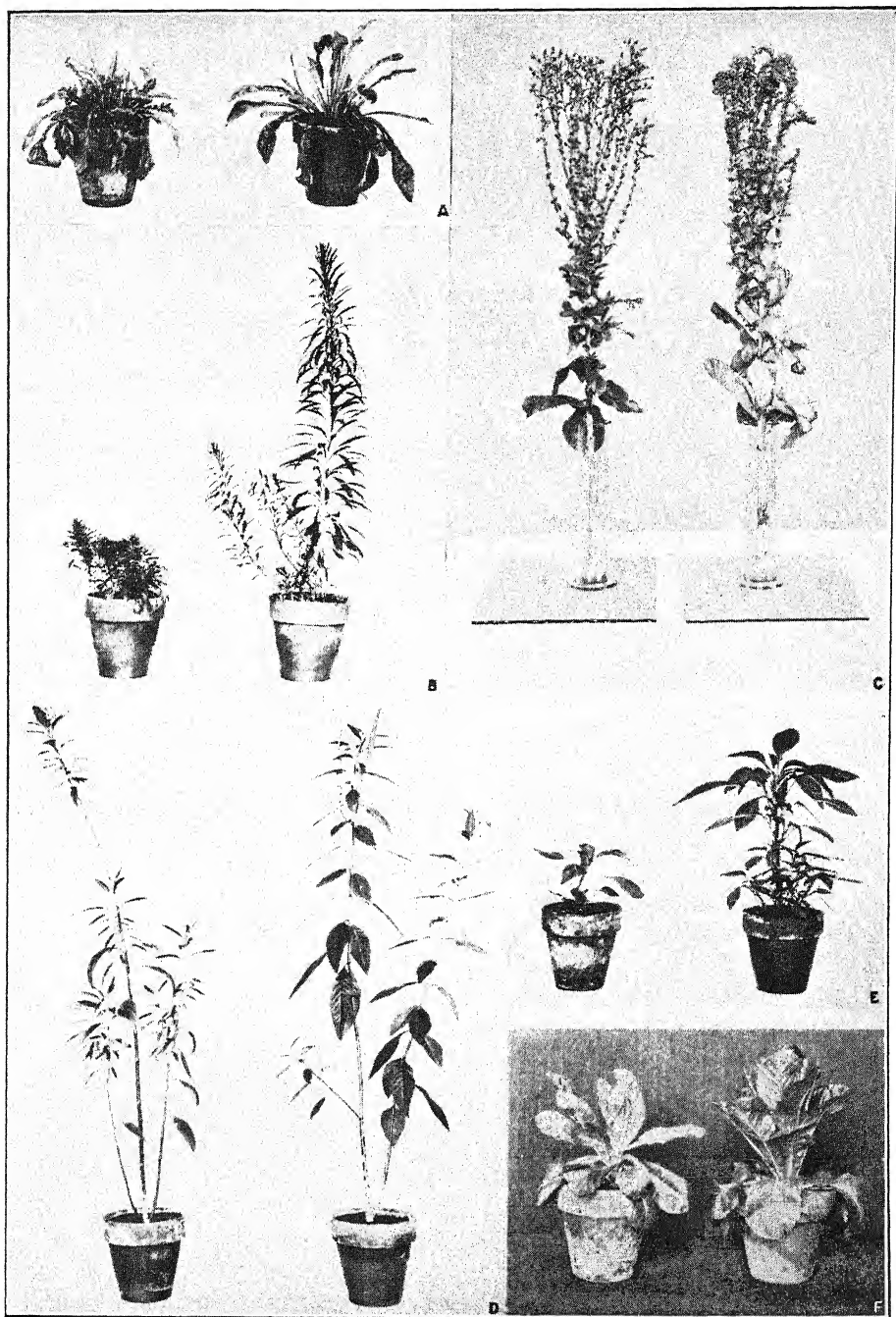
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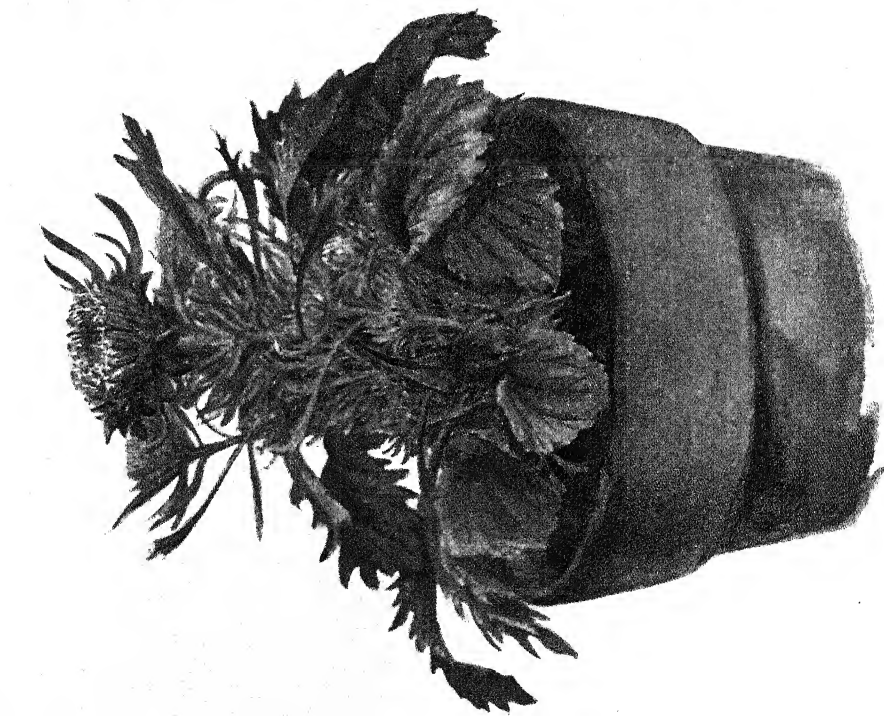
KUNKEL: ASTER YELLOWS



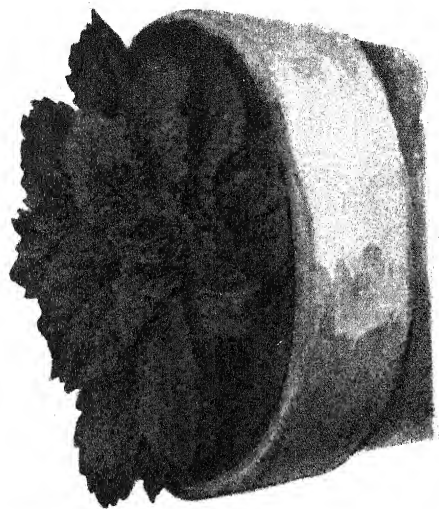


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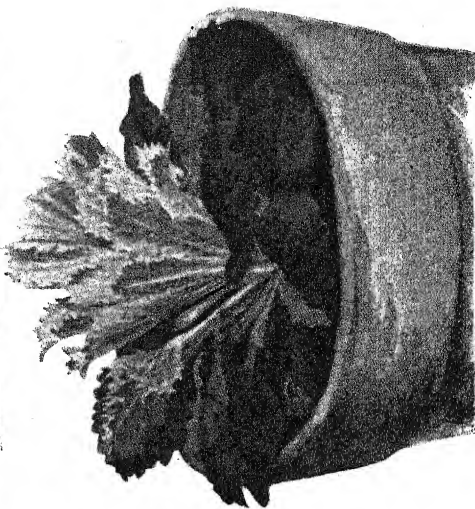




A



B



C





FIG. F. A healthy and a diseased plant of *Gaillardia aristata* Pursh. The diseased plant is badly dwarfed and chlorotic. It has produced many small secondary shoots.

FIG. G. A healthy and a diseased plant of *Tragopogon porrifolius* L. The diseased plant is chlorotic and somewhat stunted. It has produced many small secondary shoots.

FIG. H. A healthy and a diseased plant of *Taraxacum officinale* Weber. The diseased plant is chlorotic and has produced many secondary shoots. It is not stunted.

FIG. I. A healthy and a diseased plant of *Centaurea imperialis* Hort. The diseased plant is very chlorotic and has produced many upright-growing secondary shoots.

FIG. J. A healthy and a diseased plant of *Reseda odorata* L. The diseased plant is slightly chlorotic and somewhat dwarfed.

FIG. K. A cluster of healthy and a cluster of diseased flowers of *Reseda odorata* L. Secondary flowers have been produced on the stigmas of many of the diseased flowers.

## PLATE XLII

### Host Range of the Aster yellows Disease

FIG. A. A healthy and a diseased plant of *Sonchus oleraceus* L. The diseased plant is badly stunted and very chlorotic. Many upright secondary shoots have been produced.

FIG. B. A healthy and a diseased plant of *Gypsophila paniculata* L. The diseased plant is very badly stunted and chlorotic. It has produced many secondary shoots.

FIG. C. A healthy and a diseased plant of *Fagopyrum esculentum* Moench. The diseased plant is slightly chlorotic and somewhat dwarfed. The petals of the flowers on the diseased plant are green in color and usually somewhat reduced in size. A large number of small flowers have been produced.

FIG. D. A healthy and a diseased plant of *Pimpinella anisum*. The diseased plant is stunted and chlorotic. It has produced many fine secondary shoots.

FIG. E. A healthy and a diseased plant of *Ambrosia trifida* L. The diseased plant is dwarfed and has produced many secondary shoots. The young leaves are slightly chlorotic.

FIG. F. A healthy and a diseased plant of *Tagetes erecta* L. The diseased plant is stunted and chlorotic. The flowers are stunted and slightly green in color.

FIG. G. A healthy and a diseased plant of *Schizanthus* sp. The diseased plant is stunted and chlorotic. It has produced innumerable secondary shoots.

FIG. H. A diseased plant of *Calandrina grandiflora* Lindl. which is badly dwarfed and chlorotic. It has produced many secondary shoots.

FIG. I. A healthy plant of *Calandrina grandiflora*.

## PLATE XLIII

### Host Range of the Aster yellows Disease

FIG. A. A healthy and a diseased plant of *Ammobium alatum* R. Br. The diseased plant is dwarfed and chlorotic. It has produced many side branches. The leaves are very narrow and have wavy margins.

FIG. B. A healthy and a diseased plant of *Erigeron annuus* (L.) Pers. The diseased plant is chlorotic and much dwarfed. Many secondary shoots have been produced.

FIG. C. Portions of a healthy and of a diseased plant of *Lactuca sativa* L. The diseased plant is shown on the right of the figure. It is chlorotic and shows an upright habit of growth.

FIG. D. A healthy and a diseased plant of *Asclepias nivea* L. The diseased plant has small, narrow leaves and has failed to bear pods. It is somewhat chlorotic.

FIG. E. A healthy and a diseased plant of *Amaranthus auroro*. The diseased plant is stunted and has failed to produce the red color typical of healthy plants.

FIG. F. A healthy and a diseased plant of romaine lettuce. The diseased plant is chlorotic and has failed to head.

PLATE XLIV

Aster yellows Disease

FIG. A. A diseased aster plant in blossom. Yellowed foliage, green-colored flowers, and upright secondary shoots are shown. The plant is badly stunted.

FIG. B. A healthy young aster plant.

FIG. C. A yellowed plant of the same age, showing chlorosis and upright habit of growth of diseased leaves.

## A PHYSIOLOGICAL STUDY OF THE EFFECT OF LIGHT OF VARIOUS RANGES OF WAVE LENGTH ON THE GROWTH OF PLANTS<sup>1</sup>

HENRY WILLIAM POPP

Contribution from Boyce Thompson Institute for Plant Research.

### INTRODUCTION

The question as to whether the different parts of the spectrum vary in their effects on plants is an old one. Experiments with plants grown under colored screens were undertaken by Tessier (10) as early as 1783. During the latter part of the nineteenth century many papers appeared dealing with various phases of the subject. Development of chlorophyll and other pigments, rate of elongation of stems, changes in internal structure, plant movement, photosynthesis, and general growth and vigor of plants under different-colored lights were all investigated.

Extensive as the work was during this period, few of the results can be considered with certainty as being directly caused by quality of light because of the failure of the majority of the investigators to take into account variations in light intensity and other factors that affect the growth of plants. With regard to the light, for instance, the only statement given in many cases is that glass of a certain color was used. Very often this color was probably not spectroscopically pure. In fact, many colored glasses, besides cutting down light intensity greatly, transmit the entire visible spectrum. It is obvious, therefore, that a mere statement of the color of the glass used tells us little about the actual quality and intensity of the light falling on the plants.

In recent years improvements in the making of glass to transmit very definite regions of the spectrum have made it possible to conduct more exact experiments in this field. In 1919, Schanz (9) reported the results of his experiments with plants grown under light consisting of definite regions of the spectrum of daylight. He arranged his plants in eight beds which were covered with various kinds of glass. In the first five beds the range of wave lengths of light transmitted was gradually decreased from the violet end of the spectrum toward the red, thus enabling him to study the effect on plants of light from which greater and greater regions of the spectrum were eliminated in the blue-violet end. In the last three beds he used combinations of colored glasses which gave predominating colors of yellow, green, and blue-violet respectively.

<sup>1</sup> Published, at the expense of the Institute, out of the order determined by the date of receipt of the manuscript.

In general, he found that plants became taller the more the short rays of sunlight were cut off from them. Maximum height was obtained under red light and minimum under blue-violet. This was true for cucumbers, Fuchsia, Chrysanthemums, Lobelia, Begonia, and Oxalis; but potatoes and red beets were weakest in yellow light, a little stronger in green light, and still larger and healthier in blue-violet light.

Chlorophyll-development in beans, soybeans, and potatoes was more rapid the more the short rays were cut off, being most rapid under red light. In lettuce, chlorophyll could not be formed in normal quantity in yellow or in green light but did develop fully under blue-violet rays. Epidermal anthocyanin of the leaves of certain plants failed to develop under any glass that eliminated ultra-violet rays. Flowers also became paler in color the more the short rays were removed.

With such plants as Fuchsia, beans, and tomatoes, the time of flowering was hastened gradually and the number of flowers and fruits increased as the short rays were cut off, but under more limited regions of the spectrum, namely, under red, yellow, green, or blue-violet light respectively, the number of flowers was greatly reduced and the time of flowering was postponed. The germination of nettle seeds was favored by the elimination of the short wave lengths.

As a general result of his work, Schanz concluded that light of short wave lengths, particularly ultra-violet rays, was detrimental to the growth of plants. He therefore recommended the use of Euphos glass, which eliminates these rays, for greenhouses.

Schanz's work was superior to that of many of his predecessors in that he knew the exact ranges of wave lengths of light used. He did not, however, make any measurements of light intensity, nor did he give us accurate information concerning temperature and other factors that no doubt varied under the different types of glass. We were thus again left in doubt as to whether his results were really caused by the quality of light.

Klebs (6), in a careful study of the developmental physiology of fern prothallia, has shown that very striking formative changes can be induced in prothallia by different parts of the visible spectrum. He also showed, however, that intensity and duration of light as well as other environmental factors may bring about similar effects, which fact demonstrates the importance of measuring or recording all these factors in any study of the effect of light on plants. None of the higher plants was used in his investigation.

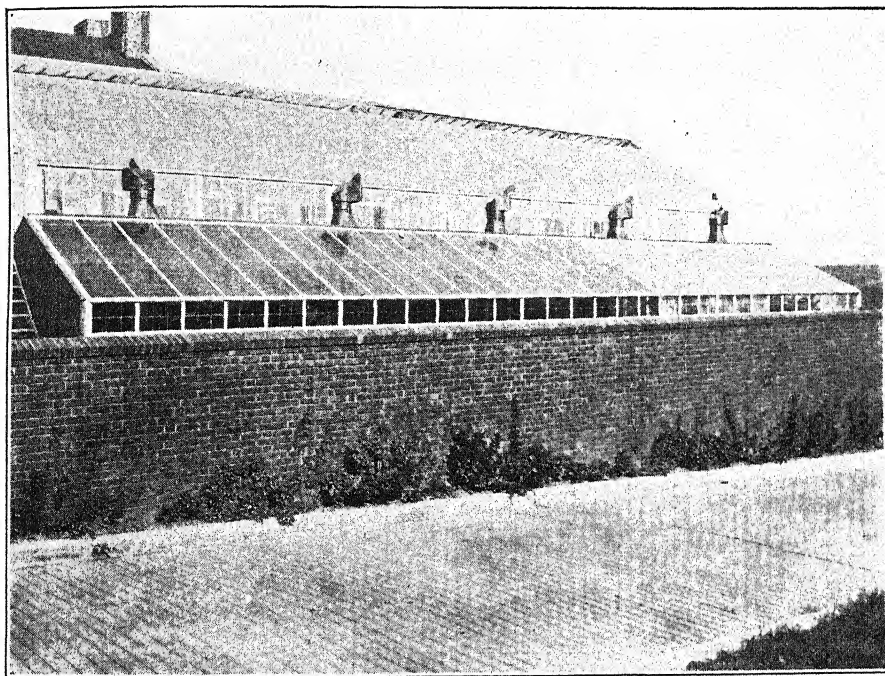
The general uncertainty of the results obtained by previous investigators and the importance of the question of light relations of plants led the Boyce Thompson Institute for Plant Research early to install such equipment as would enable workers to attack this problem under better-controlled conditions and on a scale that has hitherto not been attempted. The present paper is concerned with the results of the first investigation at this

institution on the effect of various ranges of wave lengths in the sun's spectrum on the growth of plants.

## MATERIALS AND METHODS

### Greenhouse Construction

The investigation was carried out in five small, adjacent greenhouses constructed especially for this type of work. Each house was 11 feet long and 9 feet wide. The houses had a continuous roof of glass sloping toward the south. This roof was  $3\frac{1}{2}$  feet above the benches in the front of the houses and  $5\frac{1}{2}$  feet above them behind. A photograph of these houses is given in text figure 1. For convenience in referring to them the houses are



TEXT FIG. 1. Greenhouses in which experiments were conducted. The copper ventilators shown in the picture are above the middle of each house. A recording pyrheliometer is attached to the ventilator of house 1, on the extreme right.

numbered 1 to 5, beginning at the right of the picture. A complete set of plants was also grown for comparison outside, adjacent to the five houses. This outside plot is referred to as house 6. Detailed observations of the plants in this outside plot are not included in this paper because of the great differences in general environmental conditions between it and the first five houses.

The houses were separated from each other on the inside by means of

metal partitions extending several feet below the benches, but were all intercommunicating below so as to allow free circulation of air. A large electric fan was installed underneath the benches of the east-end house (house 1), which communicated with all the houses by means of a continuous tin ventilator. Each house had two inlet pipes provided with dampers by the regulation of which the circulation of air and the temperature could be kept the same in all houses. In addition, each house was provided with a revolving copper ventilator on the north roof, as shown in text figure 1. The entire interior of each house was painted white.

### Temperature

The temperature of each house was automatically recorded by means of Cambridge and Paul electric thermographs. At any given time the temperature did not vary more than 2 to 4° C. in the different houses. The average temperatures during the growth period of the experiment for all houses are given in table 1.

TABLE 1. *Average Temperatures in Degrees C.*

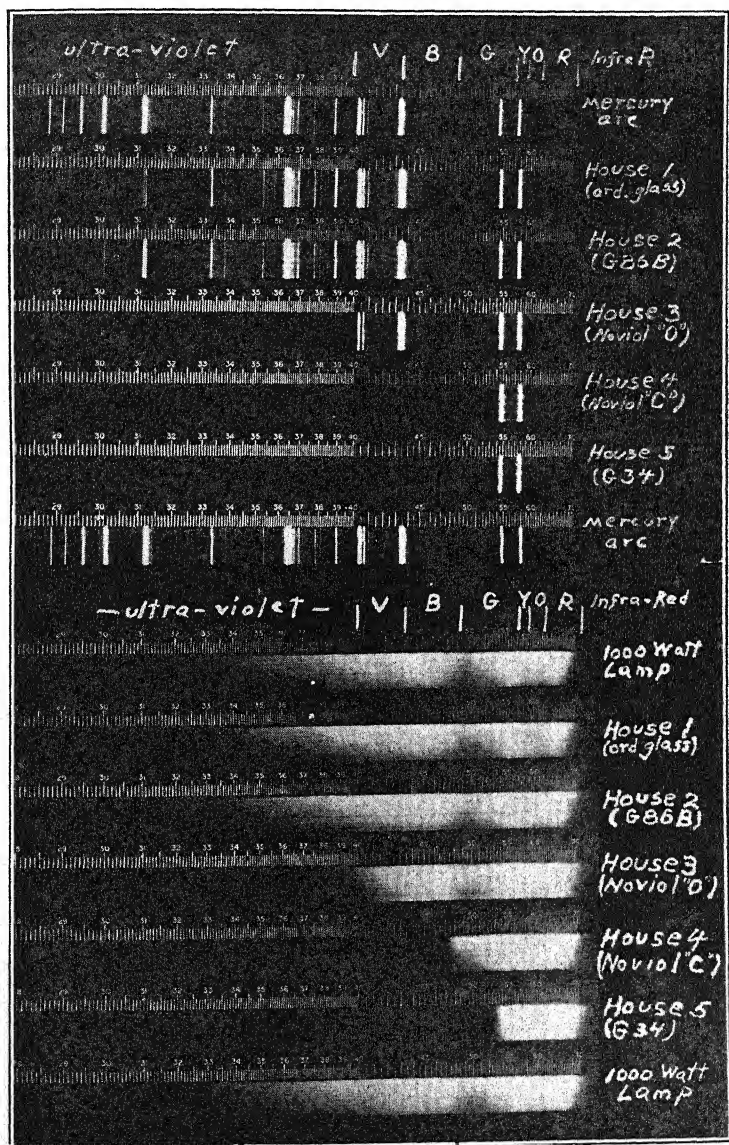
Week	Day		Night	
	Average Maximum	Average	Average Minimum	Average
Sept. 3-9.....	31.6	25.6	17.2	18.3
Sept. 10-16.....	31.1	25.0	16.7	17.8
Sept. 17-23.....	27.8	22.8	15.6	17.8
Sept. 24-30.....	28.3	23.3	13.9	16.1
Oct. 1-7.....	33.3	27.2	16.1	17.8
Oct. 8-14.....	32.2	27.2	12.2	14.4
Oct. 15-21.....	31.6	26.7	13.3	15.6
Oct. 22-28.....	31.6	25.6	17.2	18.9
Oct. 29-Nov. 4.....	28.9	25.0	18.3	19.4
Nov. 5-11.....	28.3	25.0	18.3	19.4
Nov. 12-18.....	28.9	23.3	15.6	16.7
Nov. 19-25.....	26.7	22.8	16.7	17.8
Nov. 26-Dec. 2.....	27.8	22.8	15.0	16.1
Dec. 3-9.....	25.0	21.1	16.7	17.8

The figures in table 1 were obtained by averaging the daily and hourly temperatures during the growth period. As will be seen from the table, no attempt was made to keep the temperature constant. All plants in all houses, however, were subjected to the same fluctuations of temperature, thereby eliminating temperature difference as a factor in the results.

### Light Conditions

Since it is practically impossible to maintain on a large scale an artificial light source that is comparable to daylight in both intensity and quality, the source of light used was ordinary daylight from which different parts of the spectrum were screened out by the use of different kinds of glass.

House 1 was covered with ordinary greenhouse glass and was used for comparison. The glass over the other houses was made by the Corning Glass Company for this particular work and transmitted only definite regions of the spectrum.



TEXT FIG. 2. Spectra of glasses used in greenhouses; upper series taken with a mercury-vapor arc lamp in quartz; lower series taken with a 1000-watt Edison Mazda lamp. The line at 296 millimicrons transmitted by the glass of house 2 does not appear distinctly on the print.



A quartz spectrograph was used to determine the actual range of wave lengths each glass transmitted. A print showing these ranges is given in text figure 2. Both a mercury-vapor lamp in quartz and a 200-watt lamp were used as light sources for making the spectrograms; the former because it is rich in ultra-violet rays and the latter because, having a continuous spectrum, it enabled one to determine the exact limits of those glasses the cut-off of which was in the visible region. In table 2 are given the exact ranges of wave lengths in the visible and ultra-violet transmitted by the various glasses used, together with the limits usually assigned to the different colors of the visible spectrum. It is assumed that the infra-red region begins at 720  $\mu$ .

TABLE 2. *Spectral Limits of Glasses and of Regions in the Visible and Ultra-violet*

Spectral Regions and Glasses	Spectral Range in Millimicrons ( $\mu$ )
Sunlight.....	290-720
Ultra-violet of sunlight.....	290-400
Violet.....	400-435
Blue.....	435-490
Green.....	490-574
Yellow.....	574-595
Orange.....	595-626
Red.....	626-720
Ordinary greenhouse glass (house 1).....	312-720
G 86 B (house 2).....	296-720
Noviol "O" (house 3).....	389-720
Noviol "C" (house 4).....	472-720
G 34 (house 5).....	529-720

In order to determine the relative intensity transmission of the glasses used in the different houses, the light intensities were measured inside the houses by means of a Macbeth illuminometer. House 2, which transmitted practically the entire spectrum of daylight, had to be shaded on the inside by means of an 8  $\times$  8 mesh tobacco-shading cloth to reduce its intensity to a figure comparable to that of the houses having a limited spectrum. The approximate transmission of the glasses, as given in table 3, was determined

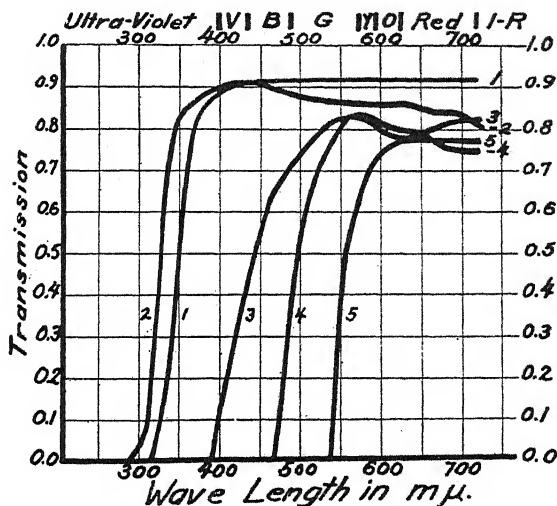
TABLE 3. *Relative Transmission of Total Intensity of Light by Glasses in Different Houses*

House Number	Approximate Transmission %	Actual Intensity 10:00-11:00 A.M. Oct. 2, 1924 (foot-candles)
Outside.....	100.0	5833
1.....	80.0	4615
2.....	46.6	2795
3.....	66.1	3756
4.....	56.7	3372
5.....	37.0	2199

by taking the average of all the measurements of actual intensities in the different houses at different times throughout the time of the experiment and

computing the transmission on the basis of the average outside intensities at the same times. In the same table is given a sample record of the actual intensities as measured with a Macbeth illuminometer in the different houses between 10:00 A.M. and 11:00 A.M. on October 2, which was a very clear day.

It is important to note here that, while the intensities were not exactly the same in the different houses, the intensity in house 2, which transmitted the entire spectrum of daylight, was at all times lower than that in house 4 and only slightly greater than that in house 5. The latter two houses, as will be seen in text figure 2 and table 2, had the narrowest ranges of wave



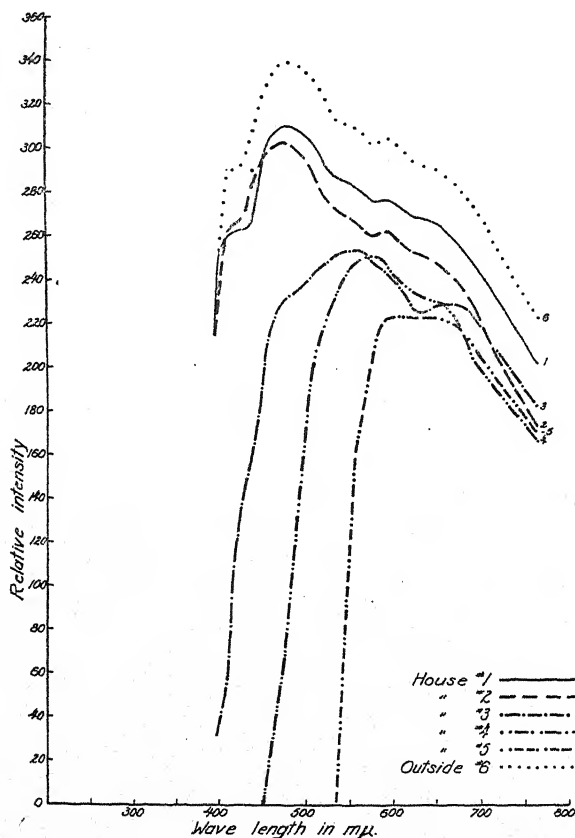
TEXT FIG. 3. Transmission curves of glasses in the visible and ultra-violet. Figures on curves represent house numbers; 1 is ordinary greenhouse glass (house 1); 2 is Corning glass "G86B" (house 2); 3 is Corning's Noviol "O" (house 3); 4 is Corning's Noviol "C" (house 4); 5 is Corning glass "G 34" (house 5).

lengths in the spectrum. It is apparent, therefore, that intensity differences could not be a controlling factor in the final results obtained in these houses.

Since the intensity of daylight varies from hour to hour and from day to day, no attempt was made to keep a constant record of it by measurements with a Macbeth illuminometer. Since, however, the exact transmission of the glasses is known, and since the U. S. Weather Bureau keeps a constant record of intensity at its New York City station, which was only a few miles away, such records are available. These records are given in gram calories per square centimeter per minute. Kimball (4) has found that if these figures (gram cal. per cm.<sup>2</sup> per min.) are multiplied by the factor 6700, the resulting figure will give the illumination intensity in foot-candles. From the latter figure the intensity in any of the houses can be computed by simply multiplying by the transmission of the glass in that

house. It is therefore possible to determine the actual illumination intensity for any given time in any house throughout the growth period.

Text figure 3 gives the transmission curves of the various glasses in all regions of the visible and ultra-violet. These curves were constructed from curves obtained for the various types of glass and corrected for the thickness of the actual glasses used. Curves showing the relative intensity of different wave lengths of light as received on a horizontal surface in the open from sun and a cloudless sky at different hours at latitude  $41^{\circ}$  N. on September 21



TEXT FIG. 4. Relative energy-distribution in the visible spectrum of the light transmitted by the glass in the different houses, as determined for noon, September 21, on a clear day. The curve for house 2 does not take into account the reduction in intensity equally at all wave lengths, caused by the tobacco-shading cloth used in this house, which causes the entire curve to be shifted to a lower position but does not change its shape.

have been made by Kimball (5). Similar curves are available for other seasons of the year, but these particular curves would hold approximately for the period during which the experiment was conducted. By applying to these curves the percentage transmission of the different glasses at

different wave lengths, as given in text figure 3, curves are obtained which give the distribution of the energy in the visible spectrum of the light transmitted by each of the types of glass. A series of such curves, determined for each kind of glass used in the different houses, for noon, September 21, is given in text figure 4.

### Plants and Culture Methods

The following plants were grown in each house: Tobacco (*Nicotiana tabacum*) variety Havana, 5 pots; carrots (*Daucus carota*) variety Danvers Half Long, 4 pots; petunia (*Petunia hybrida?*) variety Balcony Crimson, 4 pots; sunflower (*Helianthus cucumerifolius*), a small variety, 5 pots; soybeans (*Soja max*) variety Peking, 6 pots; four o'clocks (*Mirabilis Jalapa*), 2 pots; coleus (*Coleus Blumei*), variegated, 2 pots; buckwheat (*Fagopyrum vulgare*) variety Japanese, 2 pots; tomato (*Lycopersicum esculentum*) variety Bonny Best, 2 pots; Sudan grass (*Holcus sorghum sudanensis*), 4 pots.

All plants were grown in 2-gallon jars, perforated at the bottom. The soil used consisted of a top soil taken from a forest, to which was added horse manure at the rate of one part of manure to three parts of soil, and a liberal amount of potassium sulfate, acid calcium phosphate, sheep manure, and lime, the whole being thoroughly mixed. All pots were filled from the same batch of soil.

The plants were so distributed within the houses as to avoid differences resulting from shading. Whenever comparisons were made between plants in the different houses, or when photographs were taken, the plants were always taken from the same relative position in each house.

The experiment was begun on August 26, 1924. The Sudan grass, soybeans, four o'clocks, and buckwheat were planted on this day. The tobacco plants were about one inch high when placed in the houses on August 26. They had been sown August 1, 1924, and were above ground August 9. The carrots and sunflowers were sown August 22 and were not yet up when the pots were placed in the houses. The petunia plants, sown July 21, were 2 to 3 inches high, the coleus plants (July cuttings) were 2 to 4 inches high, and the tomato plants, sown July 26, 5 to 7 inches high when placed in the houses. Tobacco, petunias, four o'clocks, coleus, and tomatoes were grown one plant per pot. Sunflowers were thinned to 2 or 3 plants per pot, soybeans and buckwheat to 6 plants per pot, and carrots and Sudan grass to about 12 to 15 plants per pot.

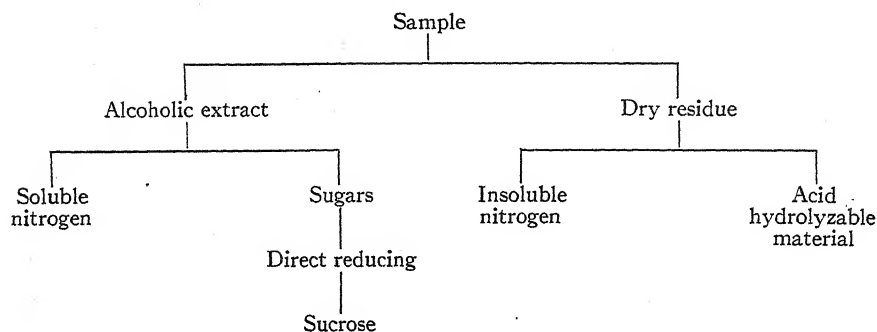
Besides general observations on vegetation, flowering, and fruiting, weekly measurements of height of all plants and chemical analyses were made, and anatomical and other differences were followed.

### Methods of Chemical Analysis

The plants to be analyzed were placed in a dark room over night and then removed to a refrigeration room and allowed to remain until frozen.

Usually they were left in the refrigeration room one to two days and never less than 3 hours. The frozen plants were then ground and mixed thoroughly. Two portions were removed to paired watch glasses to be used for moisture determinations. Two more portions, varying from 40 to 60 grams each, were placed in tared 250-cc. Erlenmeyer flasks into which one to two tenths of a gram of  $\text{CaCO}_3$  was added. These were boiled in 95-percent alcohol for 10 minutes, then made up to 200–225 cc., stoppered, sealed with paraffin, and set away in the dark until they were to be used for analysis.

The samples were extracted three times with 50-percent alcohol. The dried residue and the extract were used for determinations of carbohydrates and nitrogen according to the following outline:



Each analysis reported is the average of two separate samples carried through the complete series of analyses.

Insoluble nitrogen was run on a two-fifth aliquot of the residue, using the Official Method modified to include nitrates. Soluble nitrogen was run on a two-fifth aliquot of the alcoholic extract, using also the Official Method modified to include nitrates. Total nitrogen as expressed in the tables was obtained by adding together the results of the two preceding determinations.

Direct reducing sugar and sucrose were determined on two fifths of the alcoholic extract after removing the alcohol by evaporation, treating with saturated lead acetate, and deleading with potassium oxalate. The official Munson and Walker method was used for determining the reducing sugar, and the results were calculated as dextrose. Sucrose was determined by first calculating the results, after treatment with concentrated  $\text{HCl}$ , as invert sugar, then subtracting from this the direct reducing value and multiplying by 0.95.

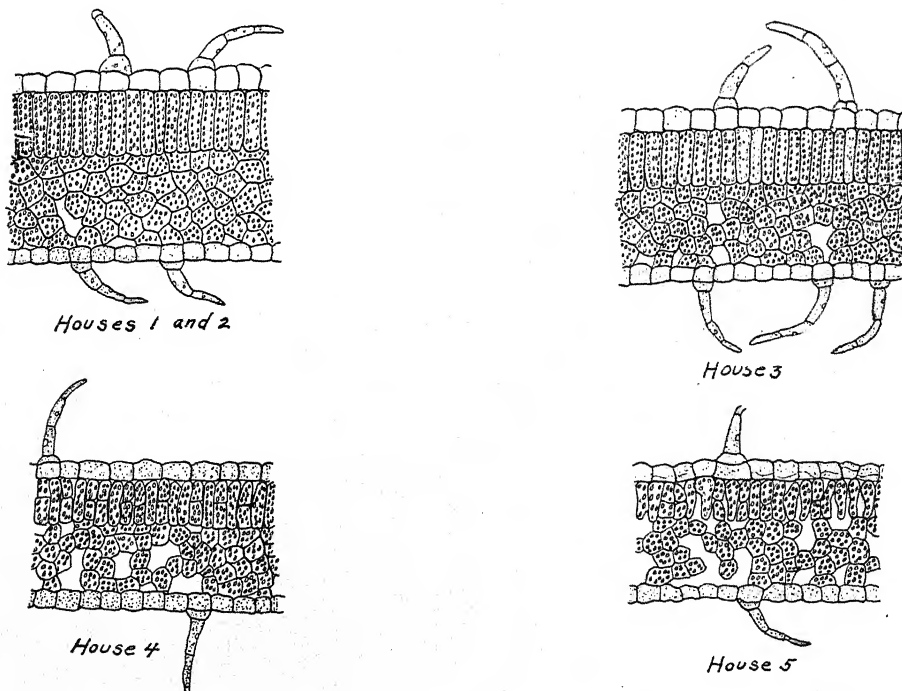
The acid hydrolyzable material was determined on two-fifths portions of the dried residue, reducing sugar being determined on the material after hydrolysis with  $\text{HCl}$ , the results were calculated as dextrose, and then multiplied by the factor 0.9.

In addition to these chemical determinations, microchemical tests for carbohydrates and nitrates were made from time to time during the progress of the work.

## RESULTS

## General Growth and Development

The most striking results in growth and development were obtained in those houses (4 and 5) in which the entire blue-violet end of the spectrum was eliminated. The plants in these houses were decidedly etiolated, although they had a good green color which in some cases, like the tomato, was even darker than that of the plants in the other houses. The stems were generally long and slender, with greatly elongated internodes and few branches. Internally these stems had a rather loose structure with little differentiation of tissues and weak development of secondary and strengthening tissues. The cells were thin-walled. Practically all the plants had to be supported after two or three weeks' growth. Soybeans completely changed their habits of growth in these houses and became twiners (Plate XLV, fig. 3). The leaves did not vary greatly in size from those of plants in the other houses, but they showed a general tendency toward crinkling or rolling which was rather pronounced in sunflower, petunia, tomato, tobacco, and four o'clocks. Leaves of tobacco were somewhat longer and narrower in these two houses. Internally, the leaves, like the stems, showed a loose structure with little differentiation of tissues. There were large air spaces.

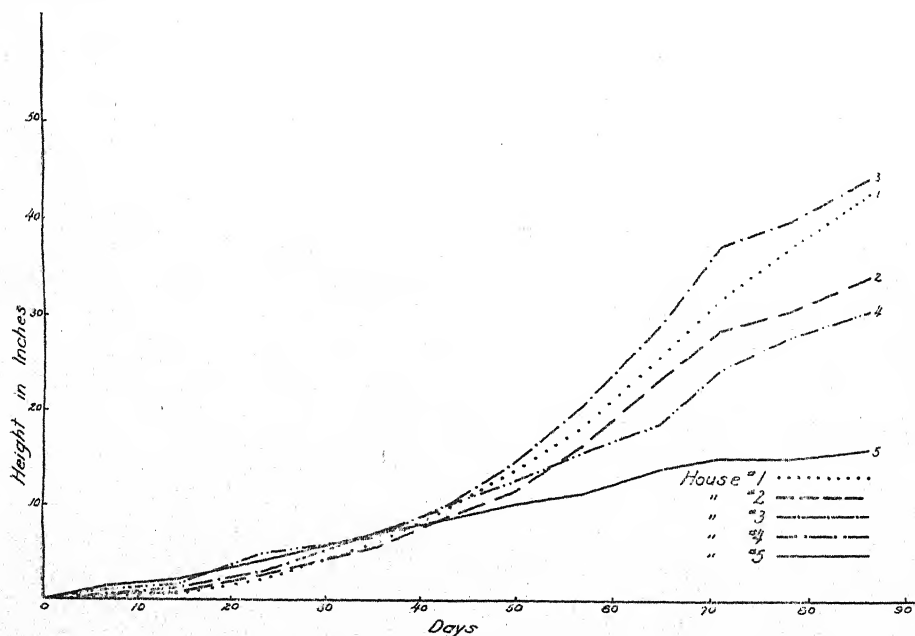


TEXT FIG. 5. Internal structure of tomato leaves in the different houses. All drawn to same scale.

The cells were thin-walled and loosely arranged even in the palisade region. Text figure 5 shows some of these features as they appeared in the tomato. The cells of the mesophyll were generally full of chloroplasts and were not greatly different in this respect from those of plants in the other houses.

The leaves of variegated coleus, which in the first three houses were of a dark red color, were much paler in houses 4 and 5. Normally there is a very narrow green margin on these leaves. This margin became much wider in houses 4 and 5, and particularly in house 5. The color of the flowers of tobacco, four o'clocks, and petunia was also somewhat paler in houses 4 and 5.

With the exception of the soybeans, all the plants in houses 4 and 5 were delayed in reaching maturity as compared with the plants in the other houses. The pods of soybeans in houses 4 and 5 began to dry out and ripen in 70 days, and then no new pods were developed. The leaves also began to turn yellow at this time. In the other houses, new pods kept forming and the plants remained vigorous and green to the time they were sampled for analysis (91 days).

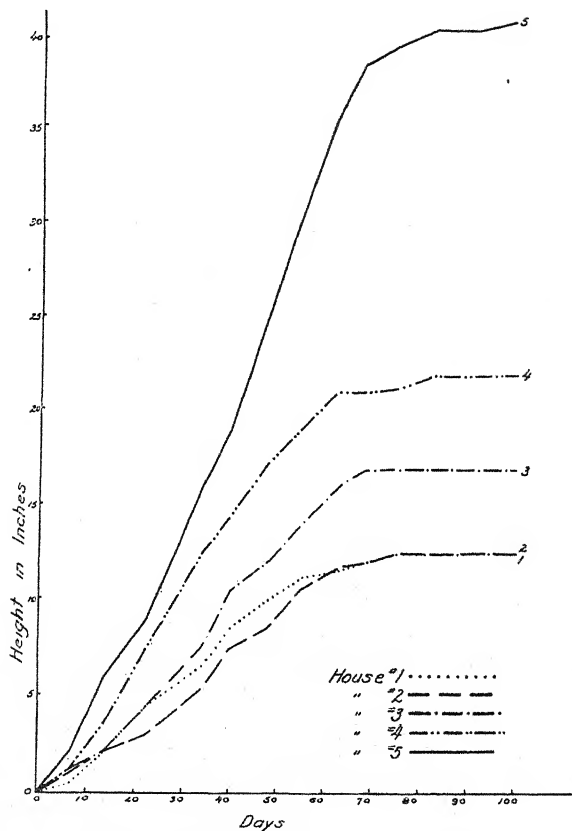


TEXT FIG. 6. Growth in height of sunflowers in the different houses.

Removal of only the ultra-violet rays (house 3) had little effect on the general growth and appearance of the plants as compared with those receiving these rays (houses 1 and 2). The plants were slightly taller and in some cases bloomed earlier, but otherwise no great differences were observed. All plants had a vigorous and healthy appearance in the first three houses.

### Stem Elongation

No differences were observed in the rate of germination of seeds in the different houses. All plants that were sown in the houses came up on the same day in all the houses. As soon as they appeared above ground, however, the seedlings began to show differences in the rate of growth. For



TEXT FIG. 7. Growth in height of four o'clocks in the different houses.

the first 2 or 3 weeks the rate of stem-elongation was uniformly greatest in all species in houses 4 and 5, which had the narrowest range of wave lengths in the spectrum (table 4 and text figures 6-8). After that time the rate in these two houses fell below that of the plants in the other houses in the cases of Sudan grass, petunia, sunflower, and buckwheat. In all these species the shortest stems at the end of the experiment were found in house 5 and the next shortest in house 4.

There was little difference in the rate of growth of tobacco in the different houses during the first 40 days. For a short period after this the plants in houses 4 and 5 fell behind those of the first two houses. It was at this time

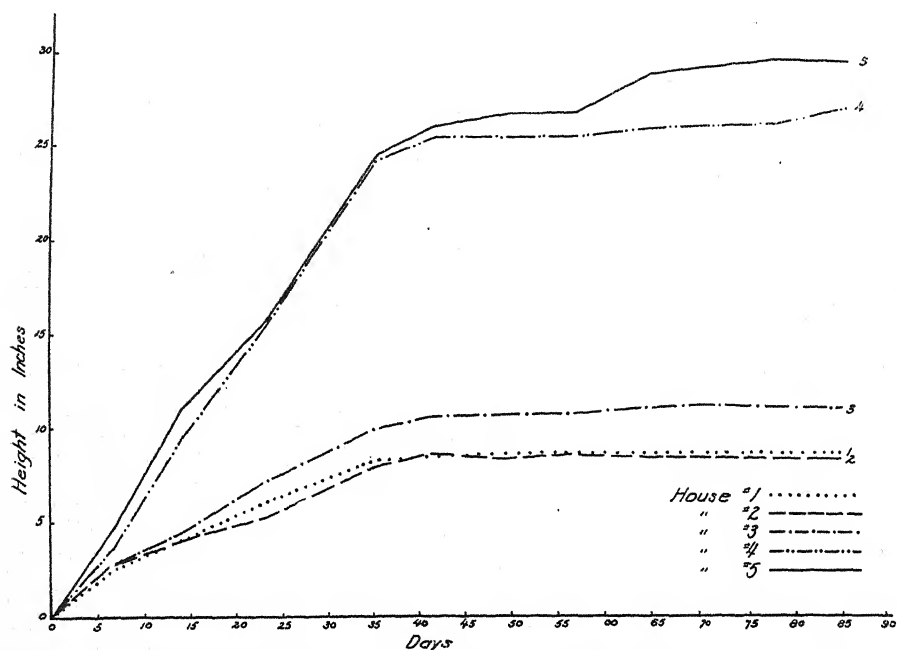


TABLE 4. *Average Height of Plants in Inches, Measured from Base of Stem to Highest Point*

Plant	House No.	Hgt. at Start	Days in Houses										No. Pots				
			7	14	23	35	41	49	56	64	70	77		85	94	102	
Tobacco.....	1	1.0	1.5	2.5	6.4	9.4	12.1	19.2	31.7	37.2	36.5	36.5	36.5	36.5	36.5	36.5	5
".....	2	1.0	1.5	2.6		9.1	12.4	20.1	32.3	39.2	38.5	38.5	38.5	38.5	38.5	38.5	5
".....	3	1.0	1.5	2.6	6.0	8.5	11.4	16.2	28.5	41.0	44.3	44.5	45.5	45.5	45.5	45.5	5
".....	4	1.0	1.5	2.6		8.5	9.7	14.3	21.8	35.5	40.3	41.5	41.5	42.0	42.0	42.0	5
".....	5	1.2	1.8	2.8	6.6	10.7	12.5	17.6	25.3	42.7	48.3	49.5	49.5	49.5	49.5	49.5	5
Sudan grass.....	1	0.0	3.0	5.2	13.5	19.9	22.4	28.5	30.7	40.2	49.1	54.1	59.0				4
".....	2	0.0	2.7	5.4	10.4	15.9	19.2	25.2	30.9	43.2	51.5	56.7	61.1				4
".....	3	0.0	2.8	5.9	11.5	19.0	22.9	28.9	36.6	46.5	53.2	60.4	61.4				4
".....	4	0.0	3.2	6.0	9.0	18.0	20.5	26.4	39.7	40.7	43.6	44.7	48.0				4
".....	5	0.0	3.5	6.2	9.7	17.6	19.5	24.0	29.1	36.9	37.2	38.5	42.5				4
Petunia.....	1	2.5	2.9	3.2	4.1	5.0	7.1	8.5	14.4	18.9	22.7	26.5	29.0	31.7	34.2		4
".....	2	2.7	3.1	3.6	5.6	7.0	9.4	12.4	15.6	20.1	22.9	27.5	28.6	31.0	31.6		4
".....	3	3.0	3.4	4.2	6.1	9.4	11.6	15.0	17.2	23.0	28.7	33.1	33.4	35.6	35.6		4
".....	4	2.7	2.8	3.7	4.7	6.2	7.7	10.1	12.7	18.4	19.7	21.3	21.7	23.7	24.4		4
".....	5	2.8	4.0	5.9	8.4	9.4	9.5	12.0	16.6	17.7	23.5	23.6	23.6	25.7	27.6		4
Sunflower.....	1	0.0	0.4	0.6	2.0	5.7	8.8	13.4	17.9	25.0	30.8	36.0	41.6				5
".....	2	0.0	0.5	0.7	2.5	5.3	8.0	11.3	16.0	22.6	27.6	29.6	33.2				5
".....	3	0.0	0.5	1.1	2.9	6.5	9.2	14.0	20.0	28.3	36.1	38.7	43.2				5
".....	4	0.0	0.8	1.5	4.9	6.5	9.4	12.3	15.2	18.2	23.6	27.0	29.7				5
".....	5	0.0	1.3	2.0	4.0	6.9	8.0	9.8	10.1	13.7	14.7	14.8	15.9				5
Tomato.....	1	6.9	8.0	10.1	14.5	22.4	26.2	32.0	37.2	43.0	43.8	43.9	44.0				2
".....	2	6.9	7.7	10.2	14.0	21.7	24.5	29.5	35.7	44.0	45.7	46.0	46.7				2
".....	3	6.2	7.7	10.3	16.5	23.9	26.7	30.8	36.5	46.5	49.7	50.5	51.0				2
".....	4	6.2	7.7	10.0	17.0	28.2	35.2	41.7	46.0	54.5	56.8	57.0	57.5				2
".....	5	6.2	9.5	15.1	23.0	37.5	40.5	49.5	54.0	54.5	59.0	59.2	59.3				2



that the plants of the first two houses were rapidly sending up a flowering shoot, which caused the height of the plants to increase rapidly for a period of about 10 days. The plants in houses 3, 4, and 5 did not flower until 5, 6,



TEXT FIG. 8. Growth in height of soybeans in the different houses.

and 14 days, respectively, later, and therefore continued to grow in length after the plants in the first two houses ceased growth altogether. At maturity the tobacco plants in house 5 were the tallest, followed by those in house 3 and house 4 respectively.

Tomatoes, soybeans, four o'clocks, and coleus (Pl. XLV) produced the tallest plants of these species in the series in houses 4 and 5. The results were particularly striking with soybeans and four o'clocks (text figures 7, 8; Pl. XLV, figs. 2, 3). The soybeans at maturity were three times as tall in houses 4 and 5 as those in houses 1 and 2, and the four o'clocks were twice as tall in house 4 and over three times as tall in house 5. This increased height was in each case due to increases in length of all internodes and not to an increase in the number of internodes, as shown in table 5.

Removal of only the ultra-violet rays of daylight (house 3) also resulted in a somewhat increased rate of elongation in all species studied. With Sudan grass, petunias, and sunflowers the tallest plants of the series were produced under these conditions, and the plants of tobacco, tomato, soybeans, four o'clocks, and coleus were all taller than those receiving the full spectrum of daylight.

TABLE 5. *Average Lengths of Internodes of Soybeans (92 Days)*  
(Average of 32 Plants in Each House)

House No.	Internodes (Length in Inches)										Total Length of Stem
	Hypocotyl	1st	2d	3d	4th	5th	6th	7th	8th	9th	
1.....	1.47	1.39	0.75	1.01	1.26	0.98	0.57	0.28	0.10	0.03	7.84
2.....	1.69	1.38	0.77	1.06	1.39	0.86	0.42	0.17	0.01		7.75
3.....	1.68	1.97	1.13	1.45	1.58	1.10	0.57	0.18	0.05	0.01	9.73
4.....	2.62	5.20	4.00	5.25	6.37	3.49	0.87	0.12	0.01		27.93
5.....	3.35	6.39	4.83	6.34	5.73	2.12	0.37	0.03			29.16

### Stem Thickness

Removal of the blue-violet end of the spectrum (houses 4 and 5) uniformly resulted in decreased stem thickness. This was true both for plants that became tallest under these conditions and for those which remained the shortest of the series. Table 6 shows the thickness of soybean stems in the different houses.

TABLE 6. *Diameters of Stems of Soybeans (92 Days)*  
(Average of 10 Plants in Each House)

Inches					
House No.	Internodes				Average of all Internodes
	Hypocotyl	2d	4th	6th	
1.....	0.133	0.244	0.176	0.117	0.167
2.....	0.144	0.263	0.227	0.137	0.193
3.....	0.133	0.202	0.168	0.118	0.155
4.....	0.112	0.104	0.058	0.064	0.085
5.....	0.074	0.061	0.043	0.036	0.054

A similar condition was found in other plants in the different houses. Thus, sunflowers of house 1 had an average stem thickness of 0.41 inch; those of house 2, 0.42 inch; those of house 3, 0.39 inch; those of house 4, 0.25 inch; and those of house 5, 0.12 inch. The stoutest stems were uniformly found in houses 1 and 2, although in general the difference between the thickness of the stems in these houses and those in house 3 was not great.

The general weakness of the stems in houses 4 and 5 necessitated the supporting of these plants long before this was necessary in the other houses. The soybeans, after being in the houses 35 days, began to twine around the supports in houses 4 and 5. No twining occurred, however, in the other houses, nor did twining occur in any other species in houses 4 and 5 in spite of the fact that many of the stems were excessively long and slender and were supported in the same manner as the soybeans.

### Flowering, Fruiting, and Storage

Removal of the blue-violet end of the spectrum had a marked effect in some species on the time of flowering of the plants, as shown in table 7. The plants of houses 4 and 5 uniformly flowered later than those of the other houses. Soybeans showed little difference in time of flowering, but all other species were markedly behind in these two houses. The sunflowers of house 5 practically failed to flower, producing only a few rudimentary flowers 71 days after the first flowers appeared in house 3.

TABLE 7. *Time of First Appearance of Flowers*  
(Arranged in the Order in which the First Flowers Opened in the Different Houses)

House No. and Plant	Date of Flowering	Days from Planting	Days in House	Days behind First	House No. and Plant	Date of Flowering	Days from Planting	Days in House	Days behind First
3 Tomato....	Sept. 16	52	21		1 Soyb.....	Oct. 1	36	36	
2 Tomato....	Sept. 24	60	29	8	2 Soyb.....	Oct. 1	36	36	0
1 Tomato....	Sept. 24	60	29	8	3 Soyb.....	Oct. 1	36	36	0
5 Tomato....	Sept. 30	66	35	14	4 Soyb.....	Oct. 2	37	37	1
4 Tomato....	Oct. 3	69	38	17	5 Soyb.....	Oct. 2	37	37	1
3 Buckwh....	Sept. 20	25	25		2 Tobacco .	Oct. 20	80	55	
1 Buckwh....	Sept. 20	25	25	0	1 Tobacco .	Oct. 21	81	56	1
2 Buckwh....	Sept. 20	25	25	0	3 Tobacco .	Oct. 25	85	60	5
4 Buckwh....	Sept. 23	28	28	3	4 Tobacco .	Oct. 27	86	61	6
5 Buckwh....	Sept. 29	34	34	9	5 Tobacco .	Nov. 3	94	69	14
3 Petunia....	Sept. 15	56	20		1 4 o'cl....	Oct. 23	58	58	
1 Petunia....	Oct. 6	77	41	21	2 4 o'cl....	Oct. 24	59	59	1
2 Petunia....	Oct. 7	78	42	22	3 4 o'cl....	Oct. 28	63	63	5
5* Petunia....	Oct. 29	100	64	44	4 4 o'cl....	Oct. 29	64	64	6
4 Petunia....	Oct. 31	102	66	46	5 4 o'cl....	Nov. 19	85	85	27
3 Sudan Gr...	Oct. 14	49	49		3 Sunflow. .	Oct. 29	68	64	
2 Sudan Gr...	Oct. 21	56	56	7	2 Sunflow. .	Nov. 3	73	69	5
1 Sudan Gr...	Oct. 27	62	62	13	1 Sunflow. .	Nov. 4	74	70	6
4 Sudan Gr...	Nov. 4	70	70	21	4 Sunflow. .	Nov. 10	80	76	12
5 Sudan Gr...	Nov. 11	77	77	28	5† Sunflow.	Jan. 8	139	135	71

\* One plant only produced 2-3 flowers which soon died and then blooming ceased.

† A few very tiny flowers were produced on one plant at this date.

Tomatoes, petunias, Sudan grass, and sunflowers flowered earliest when only ultra-violet rays were eliminated (house 3). There was little difference in time of flowering between houses 1 and 2 in spite of the difference between these two houses in light intensity.

Not only was the time of flowering delayed in houses 4 and 5, but the number of flowers produced was greatly reduced. Many of the plants produced only a few flowers. Petunias, for instance, in house 5 produced two or three flowers after being in the houses 64 days, and then ceased to bloom altogether but continued to maintain a vigorous growth. Four o'clocks also produced only three or four flowers in this house. Tomatoes, soybeans, and tobacco flowered more abundantly, but did not produce as many flowers as did the plants in the other houses.

This reduction in number of flowers resulted in a considerable reduction in fruit- and seed-production in houses 4 and 5 (table 8). Many of the flowers that did develop failed to set fruit, or if fruits were formed they were very small. The ripening of fruits in houses 4 and 5 was also greatly delayed except in the case of soybeans. These plants matured before all others, but produced only one or two seeds per pod and only a few pods. In the first three houses, on the other hand, an abundance of fruits and seeds was formed in tomatoes, buckwheat, tobacco, Sudan grass, four o'clocks, and soybeans.

Similar to the reduction in number of fruits produced in houses 4 and 5 was the development of food-storage organs. Such plants as carrots and four o'clocks, which ordinarily produce large storage roots, failed to do so in these houses (table 8 and Pl. XLVI, fig. 3). In the absence of only the ultra-violet part of daylight, however (house 3), these plants had the heaviest roots of the whole series. Microchemical tests of the stems of soybeans, tomatoes, tobacco, and sunflowers also showed much less storage of starch in these plants in houses 4 and 5 than in the other houses. The general weakness and succulence of the stems in houses 4 and 5 also indicated this. This failure in the development of food-storage organs was also shown in a preliminary series of experiments in which beets, radishes, and potatoes were grown in the different houses. In houses 4 and 5 potatoes failed to develop tubers, and the roots of beets and radishes were long and slender.

TABLE 8. *Fresh Weight of Plants*  
(Grams)

Plant	House No.	Fresh Weight per Plant			No. Plants Averaged	Days from Planting	Days in Houses	Remarks
		Tops	Roots	Fruit				
*Carrots. ....	1	77.47	131.40		4 pots	139	139	Tops drying
" .....	2	139.10	124.75		4 pots	139	139	Tops green
" .....	3	174.65	137.80		4 pots	139	139	Tops green
" .....	4	87.55	65.77		4 pots	139	139	Tops green
" .....	5	29.76	14.03		4 pots	139	139	Tops green
Four o'clocks.	1	57.00	103.75		2	137	137	1 plant only for tops; mature
" " .	2	107.50	106.00		2	137	137	Mature
" " .	3	98.00	142.50		2	137	137	1 plant only for tops; mature
" " .	4	100.00	80.00		2	137	137	Still green
" " .	5	16.75	8.25		2	137	137	Still green
Buckwheat. . .	1	15.04		2.26	7	87	87	Fresh weight is for time of setting seed (42 days)
" ...	2	12.41		2.01	6	87	87	
" ...	3	10.74		2.12	7	87	87	
" ...	4	6.09		0.53	7	87	87	
" ...	5	2.42		0.09	3	87	87	
Tomato. ....	1	765.50		440.00	1	122	91	8 green fruits
" .....	2	870.50		481.70	1	122	91	10 green fruits
" .....	3	767.50		362.50	1	122	91	10 green fruits
" .....	4	618.50		179.50	1	122	91	4 green fruits
" .....	5	658.00		278.00	1	122	91	6 green fruits

\* All weights of carrots are per pot.

## Fresh and Dry Weight

In tables 8 and 9 are given the fresh and dry weights of some of the plants grown in the different houses. Almost without exception both the fresh weight and the dry weight of the plants as a whole or of any part of the plants were lowest in houses 4 and 5. With the exception of the soybeans, the percentage of moisture in the plants in these two houses was greater than that of the plants in the other houses. The lower percentage of moisture in the soybeans resulted from the fact that these plants were drying out and were more mature at the time of sampling, all samples having been taken at the same time rather than at the same state of maturity. Their dry weight was considerably lower, however, than that of the plants in the other houses.

TABLE 9. *Fresh and Dry Weights of Plants Analyzed*

Plant	House No.	Fresh Wt. Tops per Plant (Grams)	% Moisture	Dry Wt. per Plant (Grams)	No. Plants Averaged	Days from Planting	Days in Houses	Condition when Sampled
Sudan grass.	1	18.69	71.35	5.35	16	92	92	Seeds forming
" "	2	21.28	74.77	5.37	12	92	92	Seeds forming
" "	3	20.27	72.30	5.61	13	92	92	Seeds forming
" "	4	17.31	78.83	3.66	17	92	92	Flowering
" "	5	20.53	81.70	3.76	30*	92	92	Flowering
Sunflower...	1	181.20	85.34	26.56	2	96	92	Full bloom
" ...	2	174.30	88.46	20.11	2	96	92	Full bloom
" ...	3	163.50	87.93	19.73	2	96	92	Full bloom
" ...	4	64.80	90.65	6.06	4	96	92	No flowers
" ...	5	4.32	91.67	0.36	14*	96	92	No flowers
Tobacco....	1	204.00	89.90	20.60	1	67	42	Buds forming
" ....	2	249.60	89.94	25.10	1	67	42	Buds forming
" ....	3	213.70	89.89	21.60	1	67	42	Buds forming
" ....	4	176.40	91.43	15.10	1	67	42	Buds forming
" ....	5	152.00	91.45	13.00	1	67	42	Buds forming
Tomato....	1	765.50	89.94	77.01	1	122	91	Green fruits
" ....	2	870.50	90.11	86.09	1	122	91	Green fruits
" ....	3	767.50	89.13	83.43	1	122	91	Green fruits
" ....	4	618.50	89.33	66.00	1	122	91	Green fruits
" ....	5	658.00	91.02	59.09	1	122	91	Green fruits
Soybean....	1	11.23	74.90	2.82	24	91	91	Green pods
" ....	2	9.65	73.30	2.58	18	91	91	Green pods
" ....	3	10.81	72.20	3.01	18	91	91	Green pods
" ....	4	6.48	69.40	1.98	28	91	91	Ripe pods
" ....	5	2.90	67.05	0.86	34	91	91	Ripe pods

\* The greater number of plants here was necessary in order to obtain a sufficient sample for analysis.

There was little difference in the first three houses in fresh or dry weight of plants. Tobacco, four o'clocks, tomatoes, and Sudan grass had the greatest fresh weight in house 2, which transmitted the greater part of the spectrum of daylight. Carrots, petunias, sunflower, and coleus had the

greatest fresh weight in house 3, which eliminated only ultra-violet rays. In no case were the differences very significant.

There was apparently no relation between height of plants and total fresh or dry weight. This is strikingly shown in the growth of soybeans in house 5 as compared with those in houses 1 to 3. House 5 had the tallest plants, yet the dry weight per plant was less than one third that of the plants in the first three houses. On the other hand, the sunflower plants of house 5 were the shortest of the series and had a dry weight which was less than one seventieth that of the plants in house 1.

On the basis of dry weight, the amount of growth made in all plants in houses 4 and 5 was decidedly less than that in the other houses, in spite of the fact that the light intensities in these houses were little different from the intensity in house 2. It is also interesting to note that there was little difference in weight of plants in house 2 as compared with that of plants in either house 1 or house 3, both of which had light intensities considerably higher than the intensity of the light in house 2. Apparently the intensity of light in houses 1 and 3 was above the maximum required by the plants for maximum increase in weight.

### Results of Chemical Analyses

The results of the analyses of different plants from the different houses are given in tables 10 to 14. In general, the plants in houses 4 and 5 had the highest percentages of total nitrogen and the lowest percentages of total carbohydrates. In most instances the soluble forms of nitrogen were particularly high in these houses, while the starch and other acid-hydrolyzable materials were much lower. Considerable variation was shown in the different species analyzed as regards the percentage of insoluble nitrogen and sugars. In sunflowers, all forms of nitrogen were much higher and all forms of carbohydrates much lower in houses 4 and 5. In Sudan grass, the percentages of sugars as well as of all forms of nitrogen were highest in these two houses. In tomatoes, dextrose was low in houses 4 and 5 but there was little difference in percentage of any other compounds determined in all five houses. This is probably to be accounted for by the facts that the fruits were included in the analyses, and that the weight of the fruits in the first three houses was about double that in houses 4 and 5. In both the leaves and the stems of tobacco, the percentage of all forms of carbohydrates was low in houses 4 and 5. There was little difference in any of the houses in the percentage of insoluble nitrogen in tobacco, but the soluble forms were much higher in the last two houses.

The percentages of nitrogen and carbohydrates in soybeans in the different houses did not conform to the general trend of these substances in the other plants. Soluble forms of nitrogen were lower in houses 4 and 5 and insoluble forms were slightly higher. The percentage of total carbohydrates was high also in houses 4 and 5. The reason for these differences,



TABLE IO. *Analyses of Sudan Grass, Entire Tops of Plants (92 Days from Planting; All in Houses 92 Days)*

House	Total Wt. of Tops	Wt. per Plant	Moisture	Nitrogen				Acid-hydrolyzable Material		Sucrose		Dextrose		Total Carbohydrates †	
				Insoluble		Soluble		Total*		Green	Dry	Green	Dry	Green	Dry
				Green	Dry	Green	Dry	Green	Dry						
1	299.0	18.7	71.35	.23	.79	.06	.22	.29	1.01	7.88	27.51	.92	3.18	9.28	32.36
2	255.3	21.3	74.77	.22	.87	.06	.23	.28	1.10	6.99	27.68	1.17	4.62	8.73	34.55
3	263.5	20.3	72.30	.17	.60	.05	.18	.22	.78	6.94	25.07	.88	3.16	8.46	30.55
4	294.3	17.3	78.83	.19	.91	.07	.34	.26	1.25	3.69	17.42	.93	4.39	5.27	24.88
5	616.0	20.5	81.70	.20	1.09	.09	.50	.29	1.59	4.00	21.87	.99	5.38	5.53	30.22

\* This is the sum of the preceding two columns.

† This is the sum of the preceding three columns.

TABLE II. *Analyses of Tomato, Entire Tops of Plants (122 Days from Planting; in Houses 91 Days)*

House	Wt. of Fruit	Wt. of Plant	Entire Plant	Moisture	Nitrogen						Acid-hydrolyzable Material		Sucrose		Dextrose		Total Carbohydrates †	
					Insoluble		Soluble		Total*		Green	Dry	Green	Dry	Green	Dry	Green	Dry
					Green	Dry	Green	Dry	Green	Dry								
1	440.0	325.5	765.5	89.94	.07	.69	.05	.50	.12	1.19	1.82	18.00	.27	2.58	2.02	20.09	4.11	44.78
2	489.7	380.8	870.5	90.11	.09	.88	.06	.58	.15	1.46	1.58	15.93	.22	2.54	2.05	20.43	3.85	42.75
3	362.5	405.0	767.5	89.13	.08	.78	.07	.63	.15	1.41	1.85	17.00	.30	2.82	1.87	17.16	4.02	36.98
4	179.5	439.0	618.5	89.33	.11	1.02	.05	.46	.16	1.48	2.18	19.97	.33	3.02	1.32	12.11	3.83	35.10
5	278.0	380.0	658.0	91.02	.09	.88	.07	.82	.16	1.70	1.46	16.32	.21	2.32	1.38	15.40	3.05	34.04

\* This is the sum of the preceding two columns.

† This is the sum of the preceding three columns.

TABLE 12. *Analyses of Sunflower Plants, Entire Tops of Plants (66 Days from Planting; in Houses 92 Days)*

House	Total Wt. of Tops	Wt. per Plant	Moisture	Nitrogen				Acid-hydrolyzable Material		Sucrose		Dextrose		Total Carbohydrates†	
				Insoluble		Soluble		Green	Dry	Green	Dry	Green	Dry	Green	Dry
				Green	Dry	Green	Dry								
1	362.4	181.2	85.34	.19	1.31	.03	.23	.22	1.54	1.02	6.96	.87	5.91	3.71	25.03
2	348.5	174.3	88.46	.21	1.77	.06	.51	.27	1.83	.20	1.69	.38	3.28	1.97	16.98
3	327.0	163.5	87.93	.21	1.70	.06	.54	.27	2.24	.20	1.63	.40	3.31	2.09	17.29
4	259.0	64.8	90.65	.19	2.01	.15	1.55	.34	3.56	None	None	.05	.55	.98	10.46
5	60.5	4.3	91.67	.15	1.78	.15	1.75	.30	3.53	None	None	None	None	.59	7.13

\* This is the sum of the preceding two columns.

† This is the sum of the preceding three columns.

TABLE 13. *Analyses of Havana Tobacco (Plants 67 Days Old; in Houses 42 Days; Flower Buds Forming)*

## Leaves

House	Total Wt. of Tops	Wt. of Leaves* per Plant	Moisture	Nitrogen				Acid-hydrolyzable Material		Sucrose		Dextrose		Total Carbohydrates	
				Insoluble		Soluble		Green	Dry	Green	Dry	Green	Dry	Green	Dry
				Green	Dry	Green	Dry								
1	204.0	176.6	89.74	.22	2.12	.05	.49	1.91	18.58	.17	1.67	.48	4.64	2.56	24.89
2	249.6	198.7	89.95	.23	2.34	.06	.56	2.16	21.42	.17	1.64	.36	3.70	2.69	36.76
3	213.7	180.1	89.76	.24	2.21	.07	.61	1.86	17.98	.18	1.67	.46	4.52	2.50	24.17
4	176.4	158.1	91.41	.24	2.77	.09	.99	.78	9.02	.10	1.09	.32	3.79	1.10	13.90
5	152.0	126.6	91.29	.19	2.13	.09	1.06	.42	4.85	.08	.84	.20	2.26	.70	7.95

## Stems

	Total Wt. of Stems	Wt.† per Plant													
1	63.5	31.8	.11	1.15	.09	1.03	.84	9.14	.60	6.34	1.42	15.48	2.86	30.96	
2	74.5	37.3	.11	1.03	.08	.78	1.22	11.71	.46	4.42	1.77	16.96	3.45	33.09	
3	63.6	31.8	.11	1.14	.08	.78	1.01	10.21	.60	5.42	1.69	17.20	3.30	33.83	
4	40.5	20.3	.12	1.36	.12	1.17	.82	9.09	.59	6.14	1.19	13.50	2.60	28.73	
5	47.0	23.5	.08	.97	.10	1.13	.59	7.00	.33	3.94	.68	8.03	1.60	18.97	

\* Leaves from one plant analyzed.

† Stems from two plants analyzed.

as compared with other plants, is to be found in the fact that the plants of houses 4 and 5 were much more mature at the time of analysis. Their seed pods were ripe and dry and the stems and leaves also were drying out, whereas in the first three houses the majority of the fruits were still green and the plants were succulent. The pods were all included in the analyses. Had the analyses been made at the same state of maturity of the plants rather than at the same time of growth, the results would probably have conformed to those with other plants. The total dry weight of the soybean plants in houses 4 and 5 was considerably less than that of these plants in the other houses. Soybeans were the only plants that had a lower percentage of moisture in houses 4 and 5 at the time of analysis.

The differences in percentages of nitrogen compounds and carbohydrates in plants grown in the absence of only ultra-violet rays (house 3) as compared with those grown in the presence of these rays (houses 1 and 2) were so slight as to be negligible. In some cases the highest total carbohydrates were produced in house 2 (Sudan grass and tobacco leaves). In other cases the highest total carbohydrates were produced in house 1 (tomato and sunflower), while in soybeans and tobacco stems carbohydrates were higher in house 3 than in the first two houses. Total nitrogen was higher in house 2 than in house 3 in soybeans, Sudan grass, and tomatoes, but lower in sunflowers, tobacco, and buckwheat. In no case were the differences very significant.

In general, the differences in the composition of the plants grown in the different houses were not so great as might be expected from the differences in growth habits displayed by the plants, particularly by those grown in the last two houses. There seems to be a greater reduction in absolute amounts of all substances formed rather than a marked change in the relative percentages of the different compounds as determined by analysis.

#### DISCUSSION

The results of this investigation indicate that plants require the rays in the blue-violet end of the spectrum for good, vigorous growth. Absence of all wave lengths shorter than  $529\ \mu\mu$  resulted in a condition of plants that is similar in many respects to that obtained when plants are grown in darkness or in light of very low intensity. Thus, there was at first a rapid elongation of the stem in practically all plants, and in many this continued to such an extent as to produce weak, spindly plants. There was little differentiation of tissues; strengthening tissues failed to develop well, and the plants were unable to remain erect unless supported. Leaves, though of normal size and containing an abundance of chlorophyll, were usually thin and curled or rolled. Their tissues were also less differentiated and loosely arranged. The removal of all wave lengths shorter than  $427\ \mu\mu$  yielded similar results though to a lesser degree.

We have in this case, therefore, a type of etiolation caused by quality of

TABLE 14. *Analyses of Soybeans, Entire Tops of Plants (91 Days from Planting; in Houses 91 Days)*

House	Total Wt. of Plants	Wt. of Pods	Total Wt. per Plant	Mois- ture	Nitrogen				Acid-hydrolyzable Material		Sucrose		Dextrose		Total Carbohydrates	
					Insoluble		Soluble		Total*		Green	Dry	Green	Dry	Green	Dry
					Green	Dry	Green	Dry	Green	Dry						
1	205.7	31.0	11.43	74.90	.81	3.23	.37	1.47	1.18	4.70	.66	2.63	.29	.86	5.46	21.44
2	173.6	19.2	9.54	73.30	.64	2.36	.37	1.39	1.01	3.75	.50	1.86	.30	.99	4.86	18.06
3	194.5	23.1	10.81	72.20	.63	2.27	.40	1.40	1.03	3.68	.65	2.24	.65	2.34	6.25	22.37
4	181.5	31.5	6.48	69.40	.85	2.78	.25	.82	1.10	3.60	.87	2.79	.29	1.10	7.98	25.10
5	98.7	30.7	2.90	67.05	.91	2.74	.22	.66	1.13	3.40	1.02	3.08	.28	1.08	6.88	21.08

\* This is the sum of the preceding two columns.

Note: The plants in houses 4 and 5 had many ripe seeds and were more mature at time of sampling than the others. Others had only green pods.

light rather than by decreased light intensity. That it was indeed the quality of light and not its intensity that produced this etiolation is indicated by the fact that the plants grown in house 2, which transmitted practically all the rays of the spectrum of daylight, did not have any of the characteristics enumerated, in spite of the fact that the total light intensity in this house was at all times less than that in house 4 and not much greater than that in house 5. Furthermore, there was little difference in general growth and height of the plants in house 2 as compared with those in house 1 in which the intensity was almost twice as great but the quality was about the same. In fact, in some cases the plants of house 2 were even shorter than those of house 1 and had a somewhat greater dry weight. This fact would indicate that the intensity of light in house 2, and therefore also of that in houses 4 and 5, was above the maximum requirement of the plants, and hence any increase in intensity of the light in houses 4 and 5 could not be expected to be able to overcome the characteristics of growth which the plants in these houses manifested.

It has been shown rather conclusively (Timiriazeff, 11; Lubimenko, 8, and others) that photosynthesis proceeds at a more rapid rate in the red end of the spectrum than it does in the blue-violet end. In fact, the careful work of Ursprung (12) has shown this to be true even when regions of equal energy value in the red and blue-violet regions are used. Since, in the present work, the relative intensity of the red end of the spectrum did not vary greatly in the different houses, and since the total intensity was also nearly the same in houses 2, 4, and 5, we should expect to find the rate of photosynthesis not greatly different in these houses. The percentage of carbohydrates produced, however, was uniformly lower in the houses which eliminated the entire blue-violet end of the spectrum (houses 4 and 5), markedly lower in sunflowers and the leaves of tobacco, and the dry weight was greatly reduced. Hence we must conclude either that the rate of photosynthesis was reduced, or, what is more likely from the nature of the growth the plants made, that catabolic activities were increased and that much of the food made was immediately consumed. The general failure in the development of storage organs in houses 4 and 5 and the weakness in flower- and fruit-development lend weight to this viewpoint. Furthermore, Green (3) has shown that violet and ultra-violet rays are deleterious to diastase in the leaf, while red and infra-red rays are beneficial. Agulhon (1), Chauchard and Mazoue (2), and others have shown that the short wave lengths are destructive to many other enzymes *in vitro*, although they attributed this effect largely to ultra-violet rays. If the rays in the blue-violet end of the spectrum are destructive to enzymes in the plant, the elimination of these rays should result in increased enzym action and greater catabolic activity. Actual tests in the leaves must be made, however, before the matter can be definitely settled.

The most etiolated plants of all the species grown were the sunflowers in

houses 4 and 5. These plants also showed the greatest decreases in dry weight and in percentage of carbohydrates formed. The percentage of soluble forms of nitrogen was also decidedly higher in these plants.

It is clear from the results previously stated that the removal of the blue-violet end of the spectrum down to  $529\ \mu\mu$ , or even to  $427\ \mu\mu$ , is detrimental to the plant. Schanz (9), as a result of his work, concluded that the short wave lengths of sunlight, and particularly ultra-violet rays, are detrimental to plants. His conclusion, however, was based on the fact that he obtained taller plants in many cases when these rays were eliminated. In the present work, taller plants were also obtained in some species in the absence of only ultra-violet rays as well as in the absence of the entire blue-violet end of the spectrum, but the total growth and weight made in the former case was no greater and in the latter case was much less than that of plants grown in the presence of these rays. This is well shown in the case of the soybeans, in which the tallest plants of the series, namely, those grown in the absence of the entire blue-violet end of the spectrum, had less than a third the dry weight of the shortest plants, which were grown in the full spectrum. In house 3, which eliminated only ultra-violet rays, the only observable difference in the plants was a slightly increased height and a somewhat earlier flowering in some species. There was certainly not enough difference in the plants to warrant the recommendation of glass that eliminates ultra-violet rays for greenhouses, as was done by Schanz. Furthermore, in house 2, which transmitted practically all the ultra-violet rays found in daylight, though at a somewhat lower intensity, no detrimental effects on the plants were observed. Indeed, some of the most vigorous plants in general were obtained in this house. These results are in accord with the conclusions of Kluyver (7) that it is only the shorter ultra-violet rays, which are not present in sunlight as received on the surface of the earth, that are detrimental to plants. On the other hand, these results also indicate that ultra-violet rays are not indispensable for good, vigorous growth.

#### SUMMARY

An investigation was made to determine the effect on plants of removing definite regions of the spectrum in the blue-violet end. Several widely different varieties of plants were grown in five separate greenhouses so constructed that practically all conditions except the quality of light could be kept the same in all houses. House 1 transmitted all wave lengths from the red end to  $312\ \mu\mu$ ; house 2, all wave lengths to  $296\ \mu\mu$ ; house 3 eliminated only ultra-violet rays; house 4 eliminated all rays shorter than  $472\ \mu\mu$ ; and house 5 eliminated all wave lengths shorter than  $529\ \mu\mu$ .

General observations were made on vegetative vigor, flowering, and fruiting. The height of all plants was measured weekly. Chemical analyses and microchemical tests were made, and anatomical changes were followed in some cases. The following results were obtained:

1. When plants were grown in daylight from which all wave lengths shorter than  $529\ \mu\mu$  were eliminated, they developed the following characteristics as compared with plants grown in the entire spectrum of daylight:

- (a) An increased rate of elongation of the stem of all species during the first two or three weeks' growth; a greater final height in soybeans, tomatoes, four o'clocks, and coleus, but a decided decrease in height in sunflowers, petunia, buckwheat, and Sudan grass.
- (b) A considerable decrease in thickness of stems.
- (c) A reduction in the number of branches or side shoots.
- (d) A general curling or rolling of leaves.
- (e) Good development of chlorophyll, but a reduction in anthocyanin of leaves and flowers.
- (f) Less differentiation of stem and leaf tissues, less compact and thinner-walled cells, and a reduction in strengthening tissues.
- (g) Considerable delay in time of flowering and a reduction in the number of flowers produced.
- (h) Very weak development of seeds, fruits, and general storage organs.
- (i) Decrease in fresh weight and dry weight and an increase in percentage of moisture.
- (j) Considerable decrease in starch and total carbohydrates, and generally an increase in total nitrogen; often an increase in soluble nitrogen compounds.

The degree to which these different effects were produced varied with different species, but all species, aside from the abundance of chlorophyll, had an etiolated appearance.

2. When all wave lengths shorter than  $472\ \mu\mu$  were removed, the same effects were produced as listed above, but to a somewhat lesser degree.

3. When only ultra-violet rays were eliminated, none of the foregoing results were obtained with any of the plants used, although there was a small increase in length of stems in all species except buckwheat, as compared with plants receiving these rays. Tomatoes, petunias, Sudan grass, and sunflowers bloomed somewhat earlier than they did under any other conditions. In general, there was very little difference between plants that received all the rays of the spectrum of daylight and those from which only ultra-violet rays were eliminated.

The results obtained with plants from which all wave lengths shorter than  $529\ \mu\mu$  or  $427\ \mu\mu$  were eliminated are somewhat similar to those obtained when plants are grown under greatly reduced light intensity. That light intensity was not an important factor in the present experiment is proved by the fact that normal, vigorous growth was obtained when the plants received the full spectrum of daylight at an intensity that was at all times lower than that of the house in which all wave lengths shorter than  $427\ \mu\mu$  were removed, and only slightly greater than that of the house in which wave lengths shorter than  $529\ \mu\mu$  were eliminated.

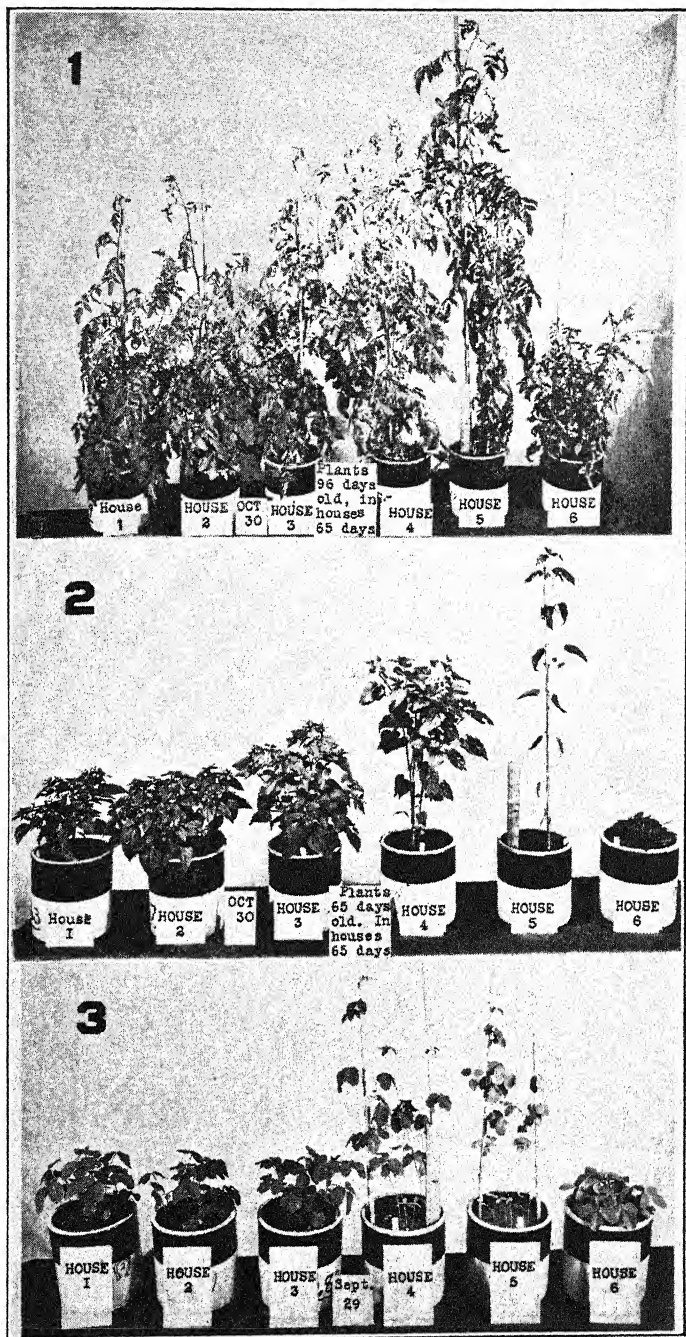


The results as a whole indicate that, while ultra-violet rays are not indispensable, the blue-violet end of the spectrum is necessary for normal, vigorous growth of plants.

The writer is much indebted to Dr. William Crocker for placing the facilities of the Boyce Thompson Institute at his disposal and for valuable suggestions and criticisms during the progress of the work; to Mr. J. M. Arthur and to Dr. Sophia Eckerson for constant interest and assistance; to Mr. James E. Webster for the chemical analyses; and to Dr. H. H. Kimball of the U. S. Weather Bureau for supplying the relative intensity curves for the various wave lengths of the spectrum of daylight, and to many other members of the Boyce Thompson Institute for helpful suggestions. He is also indebted to Dr. Charles A. Shull for his criticism of the manuscript.

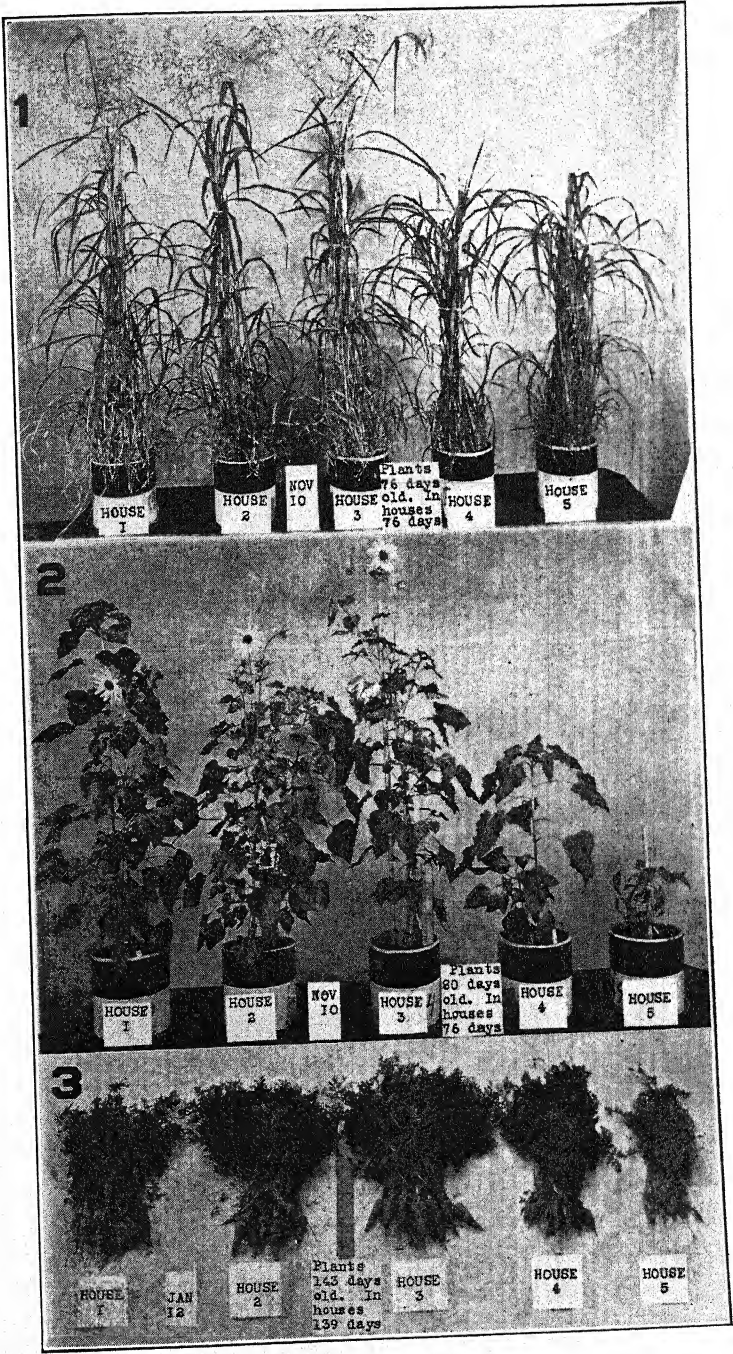
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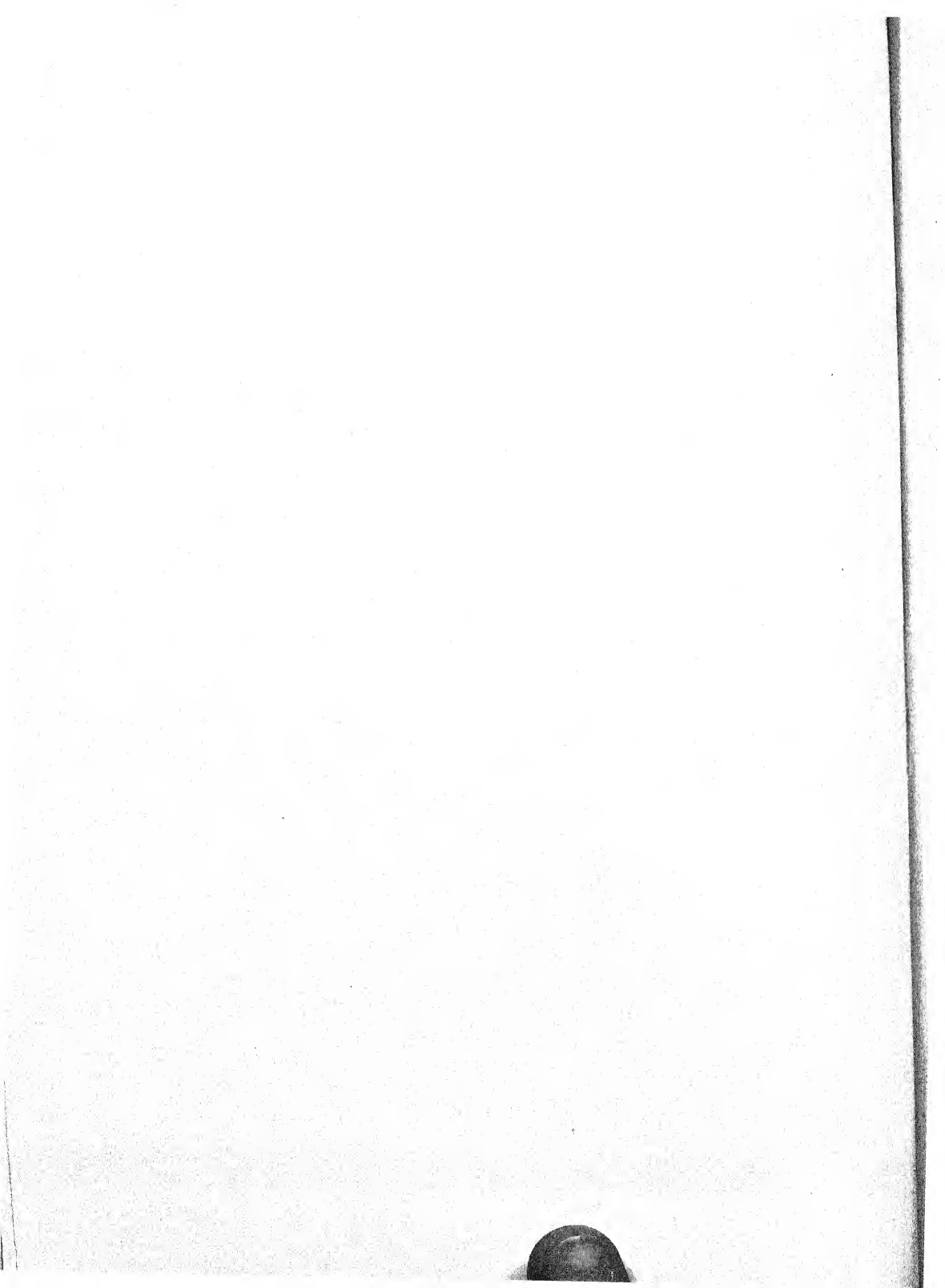


POPP: LIGHT OF VARIOUS WAVE LENGTHS





POPP: LIGHT OF VARIOUS WAVE LENGTHS



## EXPLANATION OF PLATES

In all the figures in the following plates in which "house 6" plants are shown, the reference is to plants grown in the open and used merely for comparison.

## PLATE XLV

FIG. 1. Tomatoes from houses 1 to 6, 96 days from time of planting; when plants had been in houses 65 days.

FIG. 2. Four o'clocks from houses 1 to 6, 65 days from time of planting; when plants had been in houses 65 days.

FIG. 3. Soybeans from houses 1 to 6, 34 days from time of planting; when plants had been in houses 34 days.

## PLATE XLVI

FIG. 1. Sudan grass from houses 1 to 5, 76 days from time of planting; when plants had been in houses 76 days.

FIG. 2. Sunflowers from houses 1 to 5, 80 days from time of planting; when plants had been in houses 76 days.

FIG. 3. Carrots from houses 1 to 5, 143 days from time of planting; when plants had been in houses 139 days.

## SEED GERMINATION IN THE GRAY BIRCH, *BETULA POPULIFOLIA*<sup>1</sup>

FREEMAN WEISS

Contribution from Boyce Thompson Institute for Plant Research.

The ability of *Betula populifolia* Marsh. rapidly to colonize abandoned fields and waste land is suggested in the names "old field" and "poverty" birch. In the anthracite coal fields of eastern Pennsylvania this pioneering habit is of at least some aesthetic value in initiating the forest succession on the unsightly areas that are laid waste in mining operations. Since the gray birch is regarded by foresters as a valuable nurse tree, this primary stage in the reestablishment of the forest, which here occurs without the intervention of a weed or any other successional phase, may also be of practical interest.

The areas thus denuded would appear to be extremely inhospitable even for the prevailingly xeric and acid-tolerant vegetation which, since the virtual destruction of the climax forest, covers the ridge tops of the region. In strip-mining the soil is removed down to solid rock over areas from several to many acres in extent, and the subsoil is thickly deposited over the surface soil which supported the forest. Some of the rock is pyrite-bearing and gives rise to a more acid substratum than is ordinarily considered within the tolerance of green plants. In particular, the coal washings from the breakers inundate many of the swamps and small stream valleys with the result that much of the plant life is destroyed by direct sedimentation or by the toxic action of the mine water. Though this sort of denudation is strictly local, the aggregate effect is hardly negligible. Over 3 billion long tons of anthracite have been mined from this region since 1807, and in general, one half ton of rock and soil is removed for each ton of coal.<sup>2</sup> It may be seen, then, that from 1 to 2 billion cubic yards of waste material has been deposited over the land surface within the narrow limits of the anthracite fields, occupying only about 3000 square miles. If this were deposited in a layer one yard in thickness it would suffice to cover over 200,000 acres or about 300 square miles. Of course no such proportion of the land surface is covered, as most of the culm is piled in mountainous banks. However, the data give some idea of the magnitude of the surface that is to all appearances more suitable for the colonization of gray birch than for that of any other

<sup>1</sup> Published, at the expense of the Institute, out of the order determined by the date of receipt of the manuscript.

<sup>2</sup> I am indebted to Mr. Daniel T. Pierce, vice-chairman of the Anthracite Operators' Conference, New York, for these data.



plant, and at least some stage of the succession bears this species predominantly.

What factors underlie the peculiar rôle played by the gray birch in this succession can only in part be inferred, *viz.*, the abundant production and thorough dissemination of its seed. Strong germination and high survival despite the intense insolation and seemingly precarious water supply of these bare mineral surfaces must be additional factors. Tolerance of high acidity is also important, since birch seedlings are found in pure stands in soil of pH 4 and lower, where huckleberries, blackberries, and sweet fern only tardily become established and probably only after the acidity is considerably reduced by drainage.

Germination records made by foresters would indicate that the characteristic abundance of birch reproduction is due to prolific seed-bearing and not to high germinative capacity. Toumey<sup>3</sup> cites Prussian records of germination only from 20 to 34 percent, and in his own tests reports 21 and 26 percent respectively for paper and cherry birch, with no sound seed remaining ungerminated after 100 days.

Helms and Jørgensen<sup>4</sup> observed that fresh seed of *Betula alba* L., or that preserved in dry conditions, will not germinate below 20° C., whereas that which has passed the winter on the forest floor will germinate at 10°.

Because of the interesting ecological problem afforded by *B. populifolia*, and because nurserymen and foresters are interested in the problem of seed germination in other birches of greater horticultural value, advantage was taken of a large collection of gray birch seed made at Freeland, Pennsylvania, in 1925, to carry out the study reported here. The seeds were stored dry at room temperature until January, 1926. The Davis catalase test then showed that they were viable, and an examination disclosed that 80-85 percent contained normal-appearing embryos.

Development of the ovules is slow following anthesis, which occurs early in May, and by midsummer only a minute embryo, which is apparently incapable of germination, is to be found. Toward the end of August the aments turn from green to brown and the bracts and samaras begin to loosen. The developed embryo then fills the carpel. Shedding of seed continues during the winter, the most persistent aments not being finally disintegrated until spring. Natural overwintering of the seed thus occurs mainly on the ground, but partly on the tree. A test limited to collections from two trees indicates that the seed is unable to germinate before it is fully ripe, that is, while the aments are partly green, as in early September; also that from collections made at full maturity, only low germination—at least no higher than that obtainable after a few months of dry storage—may be secured.

<sup>3</sup> Toumey, J. W. Seeding and planting in the practice of forestry. New York, 1916.

<sup>4</sup> Helms, Anna, and Jørgensen, C. A. Birkene paa Maglemose. Bot. Tidsskr. 39: 175-134. 1925. (From abstr. in Bot. Abstr. 15: 701. 1926.)



The behavior of seed subjected to dry storage for 4 months after its collection is shown in the following results from a test conducted at 32° C. At 25° germination was less than 10 percent, and at 20° almost nil.

TABLE I. *Percent Germination on Successive Dates; Started Jan. 29; in Sand*

Date.....	Feb. 3	5	8	12	15	19	Mar. 1	10	Total
Percent germination.....	16	6	12	—	1	1	3	—	39
" " .....	17	3	7	4	1	3	—	1	36
" " .....	5	8	12	5	—	1	3	—	34
" " .....	12	6	12	—	—	1	—	1	32

Various treatments applied to the seeds before sowing hastened or improved germination, but the effect was not marked in any case. Low concentrations of sulfuric acid, from  $10^{-3}$  to  $10^{-5}$  normal, whether used for a prolonged immersion before sowing, or instead of water to moisten the substratum, were somewhat accelerating. Higher concentrations were injurious, as was immersion in strong acid for over 1 minute even when followed by rinsing. More favorable results followed immersion in  $\text{KNO}_3$  for 24 hours before sowing, the effect increasing up to a N/10 solution. Ethylene chlorhydrin and sodium thiocyanate were without marked effect either way. The use of mercuric chlorid (0.1 percent solution) and of chlorophenol mercury <sup>5</sup> as a disinfectant to overcome molds proved to accelerate germination, and in some concentrations to increase the percentage of germination. Treated seeds began to germinate often in 48 hours, seed soaked only in water usually a day later, and dry seed only after 1 or 2 days more. After 5 to 10 days the difference was much less evident. Tests with a mercury hydrosol showed a similar acceleration, which accordingly was attributed to the mercury content of the materials used for disinfection. Table 2 summarizes these results.

TABLE 2. *Effect of Mercuric Disinfectants on Germination; Dry Stored Seeds on Filter Paper, 32° C.*

Treatment	Period of Treatment (Minutes)	No. of Tests	Mean Percentage Germination
Dry.....		12	42
Water.....	15-30	15	48
$\text{HgCl}_2$ , 0.1%.....	1	6	52
	5	3	40
	10	3	42
	15	5	40
	20	6	41
	25	2	42
	30	2	28
Uspulun, 0.25%.....	1	6	44
	5	3	39
	10	3	46
	15	2	53
	20	6	49
	25	2	42
	30	2	36

The percentage differences are not large and would seem of doubtful significance if repeated tests did not show rather consistently the same order. A compilation of all tests made on dry seeds or on those soaked only in water, as compared with those treated with bichlorid or Uspulun, when the exposure was not long enough to be injurious, is given in table 3. The treatments are rated numerically for each test, giving the highest award to the one resulting in greatest total germination; the ratings are then reduced to the basis of a uniform number of tests, and expressed as a percentage of the best rating.

TABLE 3. *Relative Value of Different Treatments;  
Dry Stored Seeds on Filter Paper, 32° C.*

	Dry	Water 15 to 30 min.	HgCl <sub>2</sub> 1 to 20 min.	Uspulun 1 to 30 min.
No. of tests.....	5	7	9	8
Total rating.....	5	14	19	19
Rating based on 9 tests.....	9	18	19	21
Percentage rating based on Uspulun = 100	43	86	90	100

Preliminary tests indicated that germination was best at slightly above 30° C. and that it declined rapidly both above this point and below 28°. Data obtained later are summarized below.

TABLE 4. *Effect of Temperature on Germination*

(a) Germination medium sand or peat, covered

Temp., ° C.....	15		20		25		32		10-32 (Alternating)	
	Sand	Peat	Sand	Peat	Sand	Peat	Sand	Peat	Sand	Peat
Percent germination	0	0	0	4	2	13	26	68	26	14

(b) Germination medium filter paper, not covered

Temp., ° C.....	20-26 (Variable)	25	28	32	36	38	10-32 (Alternating)
Soaked in water 15 min.....	3	5	28	40	6	0	25
Soaked in Uspulun 0.25%, 20 min.....	12	10	44	40	4	0	43

No consistent differences in germination obtained in granulated peat or sand or on filter paper were noted; for reasons of expediency, therefore, the procedure was generally followed of placing the seed on moist filter paper in Petri dishes. A covering of filter paper greatly reduced germination.

The high optimum temperature for germination of seeds kept in dry storage is worthy of special note in view of the behavior of seeds subjected to stratifying at low temperature now to be shown.

Samples of about 16,000 seeds were placed in after-ripening conditions on March 3, 1926, by mixing them with thoroughly moistened granulated

peat and storing them in constant-temperature chambers at 0°, 5°, and 10° C. The peat was not neutralized and was of about pH 4.6. One lot at each temperature was treated for 15 minutes in a 0.25 percent solution of Uspulun. After 1 month, and at regular intervals thereafter, samples of 50 seeds were withdrawn for germination tests. These were made usually on top of filter paper in petri dishes, but peat also was used. Later additional lots were stratified to test the effects of different concentrations and periods of exposure to Uspulun and other disinfectants. The results are summarized in tables 5-7.

TABLE 5. *Effect of Disinfection of Seeds before Stratification on Germinative Capacity (Mean Percentage Germination in 6 Tests)*

	Temperature During Germination, ° C.			
	15	20	25	30
Untreated.....	46	61	58	59
Treated with 0.25% Uspulun, 15 min.....	41	64	71	68

It is to be seen that a distinctly higher percentage of germination is secured from after-ripened seeds than from those kept in dry storage; that disinfection prior to stratification in general improves germination; and that there has been a downward shift in the temperature requirement; thus, germination of after-ripened seeds at 15° is equal to that of dry seeds at 32° C.

TABLE 6. *Effect of Temperature during After-ripening on Germination Percentage*

	Temperature During After-ripening, ° C.		
	0	5	10
Average of all tests started March 3.....	65	52	56
Average of all tests started May 3.....	46	54	54
Mean.....	55	53	55

The three storage temperatures employed seem to be equally effective in promoting after-ripening, although individual records indicate that at 0° C. the process is somewhat more rapid, at least during the first month. It appears, however, that disinfection at concentrations and exposures which do not impair germination at higher temperatures causes injury at 0°. Thus mercuric bichlorid, 0.1 percent, used on seeds subsequently stored at 0°, almost completely killed the lot, but with storage at 10° only slightly reduced germination. Uspulun at 0.5 percent caused no injury but was no more effective in improving germination than at 0.25 percent. Exposure to either concentration for 1 hour reduced germination as compared with exposure for 15 or 30 minutes.

TABLE 7. *Time Required to Complete After-ripening*  
*(Percentage Germination on Successive Dates at 2 Temperatures)*

Time After-ripened	Temperature During Germination, ° C.	
	20	25
30 days.....	50	51
55 days.....	62	64

An attempt to continue this observation was frustrated by germination at the storage temperature. Of the lots put in storage on March 3, those at 10° showed abundant germination *in the storage vessel* at 4 months, and those at 5° slight germination at this time. The samples stored at 0° had germinated to an amount estimated at 30 percent 6 months after placing in storage. It is of interest here to recall the practical failure of these seeds to germinate below 20–25° before after-ripening, and the observations of Helm and Jörgensen on naturally after-ripened seeds. This ability to germinate at the temperature of melting ice is doubtless a factor in the boreal distribution of birches generally, the gray birch ascending to latitude 47° in North America, and dwarf varieties of *B. pendula* penetrating the Arctic Circle.

A greenhouse planting test yielded a somewhat smaller percentage of seedlings than the germination tests promised, but showed the practicability of securing at least a 50-percent stand from fully after-ripened seed.

#### SUMMARY

The germination of *Betula populifolia* is greatly improved by storing the seeds in moist granulated peat for about 2 months at low temperature. A temperature of 10° C. is as effective for this purpose as one of 5° or 0°.

The germination percentage is increased by treating the seed with an organic mercury disinfectant before stratifying.

After-ripening results in a marked downward shift in the temperature requirement for germination.

## FACTORS INFLUENCING THE pH EQUILIBRIUM KNOWN AS THE ISOELECTRIC POINT OF PLANT TISSUE<sup>1</sup>

W. J. YODEN AND F. E. DENNY

Contribution from Boyce Thompson Institute for Plant Research.

### INTRODUCTION

One of the methods used by Robbins and his co-workers (6, 7) for determining the isoelectric point of plant tissue consisted in placing pieces of the tissue in a series of buffer solutions, and in subsequently determining the change in hydrogen-ion concentration undergone by the buffer. The pH of the buffer solution that remained unchanged in  $[H^+]$  after a period of contact was designated as the *isoelectric point*. Thus, potato tissue placed in a 0.001 *M* buffer solution with pH of approximately 6.4 remained in equilibrium with the buffer, so far as  $[H^+]$  was concerned; but if the buffer was more acid than pH 6.4, its pH was changed in an alkaline direction toward 6.4, and if more alkaline than 6.4 its pH was changed in an acid direction toward the same point.

Amphoteric substances produce a similar effect upon solutions with which they come in contact; at hydrogen-ion concentrations more acid than the isoelectric point, they unite with anions, thus changing the pH in an alkaline direction; in solutions more alkaline than the isoelectric point, they unite with cations, thus changing the pH in an acid direction; at the pH of the isoelectric point they unite with neither cations nor anions and produce no change in pH (Loeb, 4).

In this respect, therefore, the tissue showed the properties of an ampholyte, and behaved in a manner analogous to that of a protein with a definite isoelectric point.

In using this method to determine the isoelectric points of a number of tissues, we obtained results which show that the equilibrium is not caused mainly by the amphoteric character of the tissue, and that the change in hydrogen-ion concentration is not due to differential absorption of ions by tissue substances that are positively or negatively charged according to the  $[H^+]$  of the solution in which the tissue is placed.

When pieces of plant tissue were placed in buffer solutions with a graded series of pH values, the equilibrium point was approximately the pH of a water extract of the tissue made under the same conditions and for the same length of time. Most of the effect upon the buffer was not due to

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absorption of ions from the buffer by the tissue, but was caused by substances leaching out of the tissue into the buffer. By comparing titration curves of the water extract of the tissue as originally obtained and after various treatments, it was found that the substances mainly responsible for this effect dialyzed readily through collodion, were not coagulated by heat, and were soluble in acid alcohol. This shows that proteins or other amphoteric colloids can not be the main factors in causing this change in reaction on the basis of which an isoelectric point for the tissue has been postulated.

#### THE RELATION OF THE pH OF A WATER EXTRACT OF A TISSUE TO THE SO-CALLED ISOELECTRIC POINT

In carrying out the experiments to determine the effect of a given tissue upon buffer solutions, a definite weight of tissue (usually 40 grams) was placed in a bottle, and a measured amount of a 0.002 *M* phosphate, phthalate, or borate buffer solution (usually 40 cc.) was poured on. The bottle was corked and rotated, end for end, on a turning bar. To a similar amount of tissue the same amount of water was added, and this check lot was treated in the same manner and for the same length of time. At the ends of different time intervals, a sample of liquid was removed from each bottle and the pH was determined by the quinhydrone method (1).

The potato and apple tissues used consisted of thin slices about 1 cm. in diameter by 1 mm. in thickness; the potato and barley roots were cut into pieces about 3-5 mm. long; the corn, rye, and wheat seeds were whole seeds, air-dry, except in one case in which corn-seed powder was used.

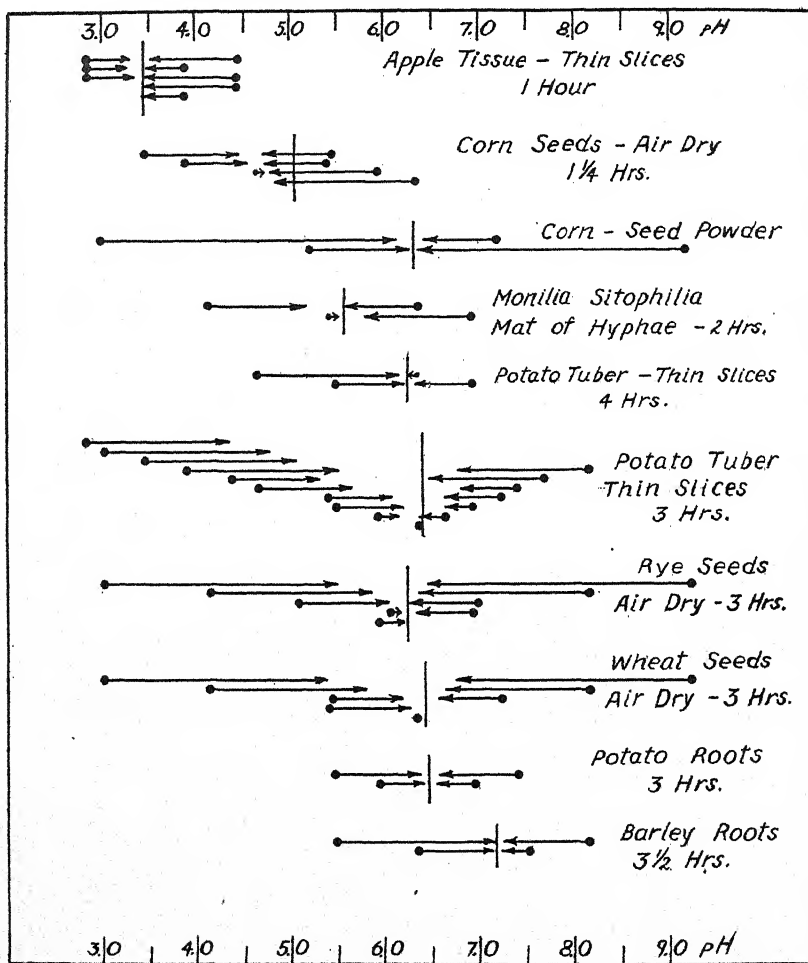
The results of these experiments are shown in text figure 1. The black dots show the pH of the buffer solution in which the sample of tissue was placed, the arrows show the direction of change undergone by the buffer, and the arrow-head shows the final pH value.

It will be noted that in all cases but one the arrows are directed toward a certain pH value, and that this value is the pH of the water extract of the tissue made under the same conditions.

Corn (*Zea mays*), air-dry seed (variety Long's Yellow Dent), as is shown in text figure 1, proved to be a possible exception among the tissues examined. The pH of the water extract of the corn seeds was not identical with the equilibrium point shown by experiments with buffer solutions. The isoelectric point found in this way after one hour's contact with buffers was always about 0.5 of a pH unit on the acid side of the pH of the water extract. When the contact was maintained for about 5.25 hours the divergence in value was reduced to about 0.25 of a pH unit. It is not known whether on prolonged contact the two values would finally become equal; bacterial action soon becomes evident if the seeds and solutions are not sterilized, and if sterilized material is used the effect of the sterilization process itself would need to be studied.

When, however, the whole corn seeds were ground to a powder, and when

samples of this powder were placed in buffer solutions and in water, the final equilibrium in the buffers was identical with the pH of the water extract of the powder, as shown in text figure 1.



TEXT FIG. 1. The relation between the pH equilibrium of tissues in a series of buffers and the pH of the water extracts of these tissues. The arrows show the pH of the buffer in which the sample of tissue was placed, the direction of change in pH, and the final pH attained. The vertical lines show the pH of the water extracts of the tissues. It is seen that the isoelectric point of the tissue as determined by this method coincides with the pH of the water extract in all tissues except in the case of corn seeds, air-dry whole seeds.

With the possible exception of whole corn seeds, therefore, the numerical value of the isoelectric point of the tissue coincided almost exactly with the pH of a water extract of the tissue. If the isoelectric point of tissue could be determined by the equilibrium point in a series of buffers, a more con-

venient method, at least for the tissues so far examined, would have been to omit entirely the use of buffers and to determine merely the hydrogen-ion concentration of the water extract of the tissue.

#### RELATION OF EQUILIBRIUM POINT TO THE HYDROGEN-ION CONCENTRATION OF PLANT JUICE

Since the so-called isoelectric point for most of the tissues examined was found to be numerically equal to the pH of a water extract of the tissue, we may inquire whether it is also identical in value with the hydrogen-ion concentration of the plant juice.

It is necessary to distinguish between the pH of the plant juice and the pH of the water extract that is obtained by bringing pieces of tissue in contact with water for certain short periods. In some cases these two values are nearly equal; thus, in the case of apple, the press-juice gave a pH of 3.39 and the water extract of the tissue after 1 hour's contact gave a pH of 3.44. In the case of potato the corresponding values were: juice = 6.10, water-extract = 6.41. With field corn, however, whole seeds rotated for 1 hour gave a water extract whose pH was 4.95, but if the seeds were ground to a powder the water extract after one hour's treatment had a pH of 6.31.

The hydrogen-ion concentration of the juice of the plant influenced the equilibrium point of the tissue in a series of buffers; but an important factor may be the rate of leaching for different substances, depending on the size of the pieces used and upon their porosity. It is likely that for powdered or porous tissues the pH of the juice of the tissue, of the water extract of the tissue, and the isoelectric point as shown by the equilibrium pH will be found to be approximately the same.

#### COMPARISON OF THE EFFECT OF THE TISSUE ITSELF AND OF A WATER EXTRACT OF THE TISSUE UPON THE REACTION OF A BUFFER SOLUTION

In order to determine the amount of change in reaction of the buffer solution produced by the tissue alone, the total change caused by tissue plus extract was compared with the change caused by extract only.

Forty grams of potato tissue were placed in each of 4 large test tubes; to 2 of these buffers were added, 1 buffer being on the alkaline side of the isoelectric point and the other on the acid side. In order to keep the volume relation comparable, 1.6 cc. of a 0.05 *M* buffer solution was added to 38.4 cc. of water; this made the solution 0.002 *M* in concentration; the pH of the diluted solution in each case is shown in column 2, table 1. To the second pair of test tubes 38.4 cc. of water only was added; the test tubes and contents were then rotated for 1 hour and the liquid was poured off; the corresponding amounts of the buffer solutions were then added to the two tubes containing the water extracts; the liquids poured off were made up to



40 cc. by rinsing with small portions of water; the final pH values were then determined.

TABLE I. *Comparison of Effectiveness of Potato Tissue and of Tissue Extracts upon the Change in pH of Buffers*

Tissue or Extract	Original pH of Buffer	Final pH of Buffer	Equivalent Amount of Acid or Alkali Required for Observed Change in pH	$\frac{\text{Extract}}{\text{Tissue}} \times 100$
Tissue.....	5.49	6.21	2.14	
	7.64	6.66	4.30	
Water extract.....	5.49	6.17	2.08	97
	7.64	6.90	3.30	77
Water extract boiled and filtered.....	5.49	6.22	2.16	100
	7.64	7.12	1.90	44
Substances soluble in acid alcohol.....	5.49	6.08	1.92	90
	7.64	7.24	1.60	37
Substances precipitated by $\text{AgNO}_3$ .....	5.49	5.85	1.40	66
	7.64	7.17	1.68	39
Substances passing through collodion.....	5.49	6.11	1.98	92
	7.64	7.08	2.12	49

The two series differ in one important respect: in one, the buffer had been in contact with the tissue for 1 hour; in the other, the buffer had not been in contact with the tissue itself, but only with the water extract of the tissue. The results are shown in table I, from which it is seen that the tissue changed the reaction of a buffer solution from pH 5.49 to pH 6.21, while the water extract changed the reaction of the same buffer from pH 5.49 to pH 6.17. From a titration curve of the same buffer solution were read off the amounts of alkali required to change the buffer from pH 5.49 to pH 6.21 or 6.17. The amounts required are shown in column 4, table I, from which it can be seen that on the acid side about 95 percent of the acid or alkali equivalent represented by the change in reaction undergone by the buffer was furnished by the water extract. On the alkaline side the tissue was more effective, furnishing about 25 percent of the total effect.

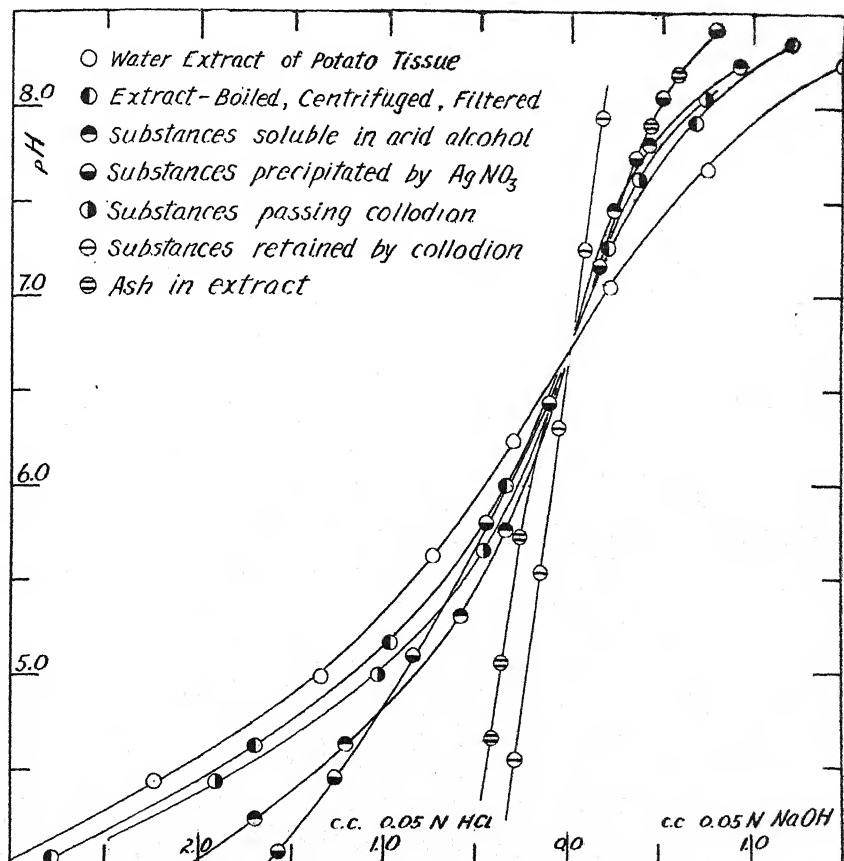
From these results we conclude that the tissue itself takes only a small part in causing the changes in the buffer solutions, that preferential absorption of ions by a tissue with a definite isoelectric point does not take place to any great extent, and that the principal cause of the change in reaction giving the appearance of an isoelectric point is related to the buffer substances that leach out from the tissue into the surrounding solution.

#### TITRATION CURVES OF THE WATER EXTRACT OF POTATO TISSUE

Since it has been shown that the substances extracted by water from the potato tissue cause most of the change in reaction of the buffer solutions coming in contact with the tissue, titration curves were made showing the

buffering capacity of the water extract (1) as obtained from the tissue and (2) after being treated in various ways. The titration curves so obtained are shown in text figure 2. In examining these curves, it should be remembered that the flatter, or more horizontal, the curve is, the greater the buffer action. A liquid having little buffer action gives almost a vertical line from pH 4 to pH 8.

From the curves shown in text figure 2, it is seen that boiling and filtering the water extract does not affect its buffer capacity on the acid side but



TEXT FIG. 2. Titration curves of the water extract of potato tissue (a) as obtained from the tissue, and (b) after the various treatments described on the graph. Note that the buffer capacity of the extract was only slightly changed by boiling and filtering, and that the principal buffer substances passed through collodion on dialysis.

causes a reduction on the alkaline side. This is also shown in table 1, column 5.

To obtain the substances soluble in acid alcohol, a measured portion of the water extract was acidified and evaporated to small volume; 95

percent alcohol was then added, and the evaporation was continued after further additions of alcohol; the mixture was then filtered and the alcohol was evaporated from the filtrate, water being added from time to time until the alcohol was completely replaced by water; the solution was then made up to the original volume. The titration curve of this extract containing the substances soluble in acid alcohol is shown in text figure 2. Much of the buffer effect was found to be present in this portion. From the data in table 1, it is seen that about 90 percent of the tissue effect on the acid side was furnished by substances soluble in alcohol; but on the alkaline side only about 37 percent of the tissue effect was attributable to these materials.

The titration curves of the materials that passed through the collodion in the process of dialysis, and of the materials that were held back by collodion, are shown in text figure 2. These are important in showing that on the acid side nearly all the buffer substances are present in the portion that passed through the collodion bag, thus showing that they are non-protein and non-colloidal.

The material in the water extract held back by the collodion (proteins and other colloids) had almost a vertical titration line from pH 4 to pH 8, showing that these materials exert almost no effect in causing the change in reaction of the buffer solutions. Other experiments showed that most of the buffer substances dialyzed through collodion within about 3 hours.

Since the principal protein of the potato, tuberin, is soluble in dilute salt solutions, and therefore may have been precipitated in the process of dialysis, a separate experiment using 5 percent NaCl for the dialysis liquid was carried out. In this case also, the material inside the collodion bag had negligible buffer capacity.

The curve marked "silver precipitate" represents the substances precipitated from the boiled water extract by acid silver nitrate. They are probably mainly organic acids. After the silver precipitate was obtained, a suspension of the precipitate in water was whirled rapidly by a stirrer, and hydrochloric acid was added drop by drop; silver chlorid was precipitated and the organic acids were set free; these were separated by filtration and the filtrate was made up to the original volume; 20-cc. portions were taken for the titration curve. This titration curve indicates that a considerable portion of the buffer action is due to organic acids present in the water extract. According to data in table 1, the substances precipitable by silver nitrate furnish about  $\frac{2}{3}$  of the buffer capacity of the tissue on the acid side, and about  $\frac{1}{3}$  of it on the alkaline side.

The graphs in text figure 2 and the data in table 1 show that, upon buffers more alkaline than the isoelectric point, the tissue itself produced an effect that was not accounted for, quantitatively, by any of the types of extracts obtained from it. Thus, in table 1, column 5, it is on the alkaline buffers that the extracts from the tissue failed to produce the amount of change in reaction caused by the tissue.

The effect of one class of amphoteric substances, the amino acids, has not been determined with exactness. The water extract of potato tissue showed an alpha-amino content of about 0.186 gram of nitrogen per liter. A solution of glycocoll containing a similar amount of nitrogen was made up, and a titration curve was prepared. This showed very little buffer action. It was considered that the rôle of amino acids in causing the change in reaction of the buffer solutions could not be studied satisfactorily until information regarding the amino acids present in the extract could be obtained.

#### BUFFER EFFECTS OF NON-AMPHOTERIC SOLUTIONS

In the preceding paragraphs the change in reaction of a buffer in contact with potato tissue is shown to be caused largely by the water-soluble buffer substances leaching out from the tissue. The capacity of a solution to cause a change in pH is described most accurately by a titration curve, and if two solutions have identical titration curves they can cause equal effects in changing the reaction of another buffer solution with which they come in contact.

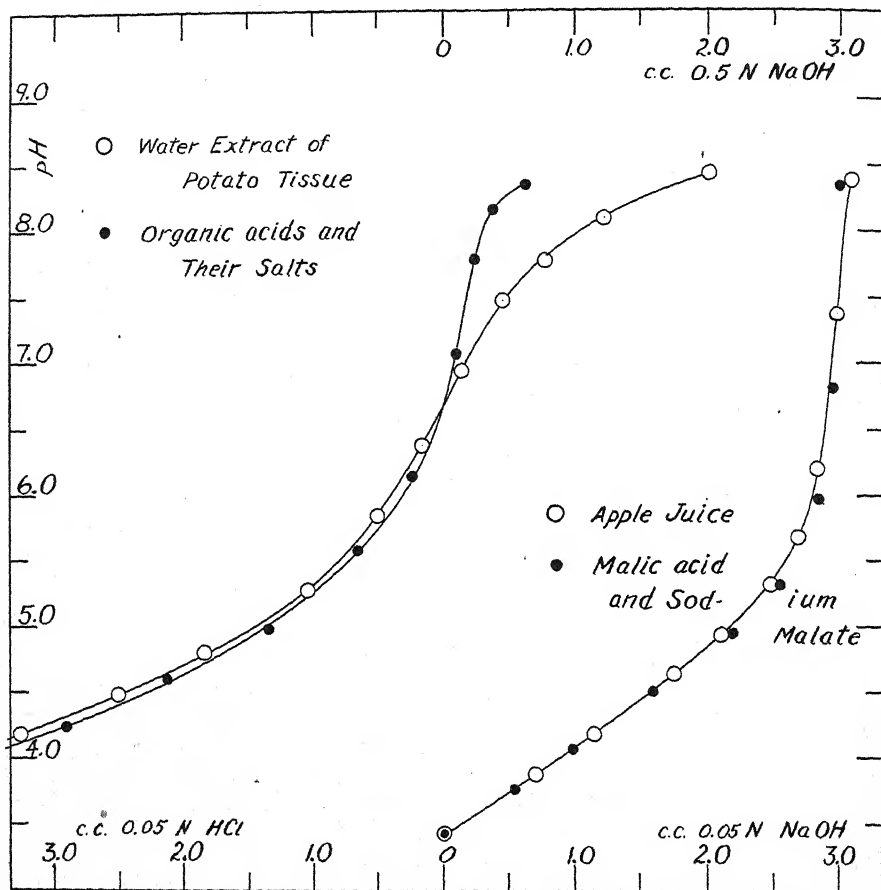
We attempted to prepare mixtures of organic acids and their salts which would give titration curves simulating the titration curves of apple juice, and of the water extract of potato tissue.

Some juice was squeezed from the pulp of Greening apples, and 20 cc. were taken for titration with  $N/2$  NaOH. The curve obtained is shown in text figure 3. To change the reaction of the juice from pH 3.43 to pH 8.39 required the addition of 3.1 cc. of  $N/2$  NaOH. An equivalent solution of malic acid was made up and found to have a pH of 2.28; sodium malate was added until the pH was 3.43, *i.e.*, equal to the pH of the apple juice. This mixture of malic acid and sodium malate was then titrated, giving the curve shown in text figure 3. The titration curves of the apple juice and of the mixture of malic acid and sodium malate were almost identical.

In another experiment, a mixture of malic, citric, oxalic, and tartaric acids having the same titration value as the water extract of potato tissue was prepared. A neutralized solution of these acids was then added to the acid solution until the mixture had the same pH as a water extract of potato tissue. This mixture was then titrated, and the titration curve is shown in text figure 3 in comparison with the titration curve of the water extract of the potato. The curves are very similar, especially on the acid side from pH 4 to pH 7. In the alkaline range the acid-salt mixture was not as well buffered as the water extract, as shown by the steepness of the curve from pH 7 to pH 8.

We do not claim that these mixtures of organic acids and salts represent the actual composition of the apple juice or potato extract. We present the data merely to show that, in order to explain the capacity of a tissue or tissue extract to cause a change in hydrogen-ion concentration in either an

acid or an alkaline direction on either side of a definite point, it is unnecessary to assume the presence of an amphoteric substance. For instance, if a portion of the artificial mixtures of organic acids and salts is added to a



TEXT FIG. 3. Similarity in the titration curves of a tissue extract or juice and of a prepared mixture of organic acids and salts. The 0.05 *N* acid and alkali refer to the potato-extract curve, and the 0.5 *N* alkali refers to the apple-juice curve.

buffer more acid than itself, the reaction will be shifted in an alkaline direction; and if added to a buffer more alkaline than itself the reaction will be shifted in an acid direction. In this respect, therefore, a mixture of organic acids and their salts behaves like a plant tissue with an isoelectric point at the pH of the acid-salt mixture.

#### DISCUSSION

It is not our purpose to claim that plant tissue does not contain substances with isoelectric points, nor that these substances are not of great

importance in the life processes of plants. Nor do we claim to have shown that the tissue itself does not have an isoelectric point. Robbins and his co-workers have brought evidence by other methods of experimentation (water absorption, toxicity of ions, staining of tissues, etc.) regarding the existence of such a point.

Our objection is mainly to the method of determining tissue isoelectric points by immersing the tissue in a series of buffers and assuming that the pH value at which no change in reaction is shown is the isoelectric point of the tissue.

Furthermore, it is unlikely that the equilibrium point of a tissue in a series of buffers represents the isoelectric point of the proteins of the tissue. Thus the equilibrium point for potato tissue is about pH 6.4; but the isoelectric point for tuberin, the principal protein of potato, is about pH 4.0 according to Cohn, Gross, and Johnson (3). Pearsall and Ewing (5) find that when the tissue is made as acid as, or more acid than, the isoelectric point of the principal protein in the tissue there is a rapid exosmosis of ions, indicating a serious injury to the tissue. The point for potato at which rapid exosmosis of chlorids took place was not at pH 6.4 but at about pH 4.4.

Chibnall (2) found that the pH of the cell contents and the isoelectric point of the cytoplasmic proteins are not identical in any tissue he studied, and points out the probability that any change in the reaction of the cell which brings the proteins to their isoelectric points will result in the death of the cell. The buffer capacity of the cell contents protects the cell against injury by tending to prevent the  $[H^+]$  from reaching the isoelectric point of the proteins of the cytoplasm.

#### SUMMARY

1. Use was made of the method of determining isoelectric points for plant tissue which consists in placing pieces of the tissue in a series of buffer solutions of varying hydrogen-ion concentrations and in noting the pH value of the buffer at which no change in reaction occurred.

2. This equilibrium pH for a number of plant tissues was found to be the pH of a water extract of the tissue in contact with water instead of buffer solution for the same time and under the same conditions.

3. Most of the effect upon the buffer solution was not due to absorption of ions from the buffer by the tissue, but was caused by substances leaching out of the tissue into the buffer. On the acid side of the isoelectric point only about 5 percent of the change in reaction undergone by the buffer was caused by the tissue itself; on the alkaline side the tissue was more effective, causing about 25 percent of the change.

4. The soluble substances which leached out of the tissue, and which exerted a dominant effect in changing the reaction of buffers in contact with the tissue, dialyzed readily through collodion, were not coagulated by heat, and were soluble in acid alcohol. This shows that proteins or other colloidal substances do not play an important rôle in causing the change in pH.

5. Since the tissue itself is not mainly involved, and since the effect produced is not due to proteins or other amphoteric colloids, it is thought that this method does not give reliable information as to the pH value of the isoelectric point of a tissue or furnish satisfactory evidence of the existence of such a point.

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## THE GERMINATION OF CENTURY-OLD AND RECENTLY HARVESTED INDIAN LOTUS FRUITS, WITH SPECIAL REFERENCE TO THE EFFECT OF OXYGEN SUPPLY<sup>1</sup>

ICHIRO OHGA

Contributions from Boyce Thompson Institute for Plant Research.

In previous papers (3, 4), I have reported the finding of very old Indian lotus (*Nelumbo nucifera*) fruits that are still alive. Thanks to their natural longevity and their impervious coats, the fruits have lain for probably more than two hundred years in moist peat in a dry, viable condition.

In the course of study of the ancient Indian lotus fruits since 1923, the writer investigated (1) the maximum and minimum amount of oxygen as related to germination and growth; (2) composition of air contained in the fruit; and (3) carbon dioxid effect on germination. The present paper is a report and discussion of these data.

### MATERIALS AND METHODS

Fruits from four sources were used in these studies: old fruits from the Pulantien Basin, South Manchuria; fruits of 1923 or 1924 from Nagoya, Japan; from Tokyo, Japan; and decorticated fruits from Shanghai, China. All showed about 100 percent germination except the decorticated fruits, which gave 44 percent germination.

Since the coats of the fruits are impervious to water, the fruits were prepared for germination by 5 hours' treatment with concentrated sulfuric acid, followed by thorough washing and later drying.

To test the relation of oxygen to the germination of the seeds, the treated fruits were placed in large-mouthed bottles filled with boiled water. The bottles were then stoppered with rubber stoppers provided with glass tubes. By proper manipulation of the tubes, the water was displaced by any desired gas. When it was desired to have the bottle practically free from oxygen, hydrogen or nitrogen gas was used for displacement, and a little vial was placed in the bottle and partly filled with potassium pyrogallate solution by an inlet tube after the boiled water had been removed from the vial by suction.

For testing the effect of known oxygen pressures on germination, treated seeds were placed in petri dishes on moist filter paper. The petri dishes were then set on tripods in large pans with a battery jar inverted over them. The pans were then filled with water, and the water was drawn to the desired height in the inverted battery jar by suction and later displaced by

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the appropriate gas. The gas was retained in the battery jar by the water seal.

To determine the composition of the gas within the epicotyl cavity of a fruit, the coat of the fruit was partly filed away and then the fruit was immersed in a vessel of mercury in such a way that a Torricellian vacuum could be applied for the withdrawal of the gas from the fruit. The gas thus obtained was analyzed in a Bonnier and Mangin apparatus (5). Each fruit gave about 0.2 cc. of gas at atmospheric pressure and room temperature. Two to ten fruits were used for each gas sample.

In order to determine the respiratory carbon dioxide liberated from dry seeds, Pettenkoffer tubes filled with NaOH were employed (2). The tests were run at 25° C., and the carbon dioxide was determined by the double titration method of Brown and Escombe (1).

Preliminary tests at 10°, 15°, 22°, 27°, 32°, and 35° C. showed most rapid germination and growth at about 32° C. Most of the experiments were conducted near this optimum temperature.

## RESULTS

### Germination in Oxygen and Nitrogen Gas

In order to determine the limit of oxygen supply for germination, the seeds, previously treated with  $H_2SO_4$  to make their coats permeable to water, were placed in bottles containing the following series of gases: pure oxygen, 4 parts oxygen and 1 part nitrogen, 3 parts oxygen and 2 parts nitrogen, 2 parts oxygen and 3 parts nitrogen, 1 part oxygen and 4 parts nitrogen, and pure nitrogen. The supply of gases was renewed every day. The results of two determinations are given in table 1.

TABLE 1. *Lengths of Sprouts of Indian Lotus Fruits in Various Proportions of Oxygen and Nitrogen*

Proportions of O <sub>2</sub> and N <sub>2</sub>	Kind of Fruits	Series I				Series II					
		Length of Sprouts* (cm.)			Average	Length of Sprouts* (cm.)					Average
Oxygen . . . . .	New	3.2	2.5	1.5	2.4	4.0	3.2	2.7	1.5	1.0	2.5
	Old	12.9	11.7	10.9	11.8	12.8	12.2	11.8	11.2	10.5	11.7
Oxygen 4 parts, nitrogen 1 part . . . . .	New	6.0	5.5	5.0	5.5	6.5	6.2	6.0	5.7	4.8	5.8
	Old	16.2	15.9	15.5	15.9	16.7	15.8	15.3	15.0	11.5	14.9
Oxygen 3 parts, nitrogen 2 parts . . . . .	New	5.3	5.2	2.5	4.3	5.8	5.2	4.7	4.2	2.7	4.5
	Old	14.7	11.5	5.4	10.5	15.5	15.2	12.8	11.6	8.4	12.7
Oxygen 2 parts, nitrogen 3 parts . . . . .	New	4.5	4.0	2.2	3.8	4.8	4.6	4.0	3.6	2.0	3.8
	Old	12.6	11.5	11.2	11.8	13.5	12.2	11.6	10.5	10.2	11.6
Oxygen 1 part, nitrogen 4 parts . . . . .	New	5.2	4.8	3.3	4.4	5.8	5.2	4.7	4.0	3.5	4.6
	Old	13.2	10.5	6.5	10.1	13.6	13.2	12.7	10.2	7.8	11.7
Nitrogen . . . . .	New	11.8	5.5	4.9	7.3	6.0	5.5	5.2	4.8	3.5	5.0
	Old	18.2	10.9	9.6	12.9	12.0	11.2	10.8	9.7	8.6	10.5

\* After 3 days at 30° C.

The results in table 1 show that: (1) the external air had little effect on the germination; (2) the sprouts made somewhat greater growth in 4 parts of oxygen and 1 part of nitrogen; and (3) sprouts from the old fruits were more vigorous than those from new ones.

### Germination at Reduced Oxygen Pressure

The germination at reduced oxygen pressure, after a week, is shown in table 2.

TABLE 2. *Lengths of Sprouts of Indian Lotus Fruits in Reduced Oxygen Concentrations*

Proportion of Gases	Kind of Fruits	Length of Sprouts after 1 Week at Room Temperature (cm.)						Average (cm.)
Air.....	New	3.5	3.2	3.0	2.7	2.5		3.2
	Old	7.2	6.9	6.8	6.6	4.6		6.4
Air 6 parts, nitrogen 4 parts .....	New	4.5	4.3	3.7	3.5	3.1		3.8
	Old	8.7	8.2	7.2	6.5	6.2		7.4
Air 4 parts, nitrogen 6 parts .....	New	4.8	4.2	3.8	2.8	2.5		3.6
	Old	8.7	7.0	6.3	5.5	5.1		6.5
Air 1 part, nitrogen 9 parts .....	New	4.9	4.5	3.2	3.0	2.0		3.5
	Old	9.0	8.3	6.2	4.8	4.7		6.6
Water.....	New	8.5	8.2	7.8	6.0	5.0		7.1
	Old	12.2	11.5	10.2	8.5	7.4		10.0

The results in table 2 show that: (1) there were no significant differences in the germination at different oxygen concentrations; (2) the fruits germinated in moist air although fruits of most water plants will not do so; and (3) the old fruits gave more vigorous sprouts than the new ones.

### Germination in Oxygen-free Condition

Six fruits were used in each test in this series. The fruit coats were removed from one end of each of three fruits in each lot, in order to facilitate the removal of the internal air in the fruits when they were later subjected to evacuation. The bottles containing the six fruits were connected to a water-suction pump giving about 400 mm. reduced pressure for 5-10 minutes, in order to eliminate as much of the gas contained in the fruits as could be removed by this amount of suction. These bottles were then filled half with water and half with nitrogen, hydrogen, or carbon dioxide gas. The fruits were immersed about 5 cm. deep in water. One bottle was filled with tap water, and another with boiled water. After a week, the results were as shown in table 3.

The results in table 3 show that when the gas that is normally present in the fruit is removed the fruits are unable to germinate, but that no other supply of oxygen is necessary for germination except that contained inside the fruit itself.

TABLE 3. *Effect on Germination Caused by the Removal of the Gas Contained Inside the Fruit*

Treatment	Air above the Water Displaced by	Kind of Fruits	Growth of Sprouts after 1 Week (cm.)			Average (cm.)
Internal air removed * . . . . .	Nitrogen . . . . .	New	Trace	0	0	17.8
Internal air not removed . . . . .		Old	Trace	0	0	
		New	16.2	17.5	19.7	
		Old	26.5	30.7	31.2	29.5
Internal air removed * . . . . .	Hydrogen . . . . .	New	Trace	0	0	13.6
Internal air not removed . . . . .		Old	Trace	0	0	
		New	19.2	20.5	1.2	
		Old	30.5	32.9	34.2	32.5
Internal air removed * . . . . .	Carbon dioxide . . . . .	New	0	0	0	20.1
Internal air not removed . . . . .		Old	Trace			
		New	20.2	22.5	17.6	
		Old	35.7	30.8	32.6	33.0
Internal air removed * . . . . .	Boiled water . . . . .	New	Trace	Trace	0	19.7
Internal air not removed . . . . .		Old	Trace	Trace	0	
		New	18.2	20.5	20.6	
		Old	30.7	32.7	31.2	31.5
Internal air removed * . . . . .	Tap water . . . . .	New	Trace	Trace	0	19.8
Internal air not removed . . . . .		Old	Trace	0	0	
		New	17.2	20.5	21.7	
		Old	30.6	34.2	36.8	33.8

\* About 1/5 of the fruit coat at the end from which the plumule emerges in sprouting was removed for the purpose of facilitating the removal of the internal air.

TABLE 4. *Analysis of Gas Contained in the Fruit of Nelumbo nucifera*

Kind of Fruits	Number of Determinations	Percentage of Carbon Dioxide	Percentage of Oxygen	Percentage of CO <sub>2</sub> + O <sub>2</sub>
Old . . . . .	6			19.41
	4			19.54
	4			19.41
	4			18.98
	4			19.31
	4	0.78	17.52	18.30
	4	0.76	18.52	19.28
	4	0.76	18.22	18.98
	4	0.73	18.22	18.95
	4	0.69	19.16	19.78
	42 Total	Ave. 0.74	Ave. 18.33	Ave. 19.11
New . . . . .	6			19.98
	5			19.48
	6			19.75
	3			19.71
	4	0.80	18.56	19.36
	5	0.85	18.55	19.40
	4	0.82	19.13	19.95
	4	0.76	19.40	20.16
	37 Total	Ave. 0.81	Ave. 18.88	Ave. 19.72

### Analysis of Gas Contained within the Fruit

The results of the analyses of the gases removed from the fruits are shown in table 4.

The results in table 4 show that there is little difference in the composition of gas obtained from the ancient and from the new fruits.

### Water Content of the Dry Fruits and the Rate of Respiration

The water content of the fruits was found to be as follows: old fruits 12.38 percent, new fruits (average of two determinations) 12.5 percent. A current of air was passed over dry fruits continuously for a period of 5 days. There was no measurable amount of carbon dioxid absorbed by the solution of sodium hydroxid.

### SUMMARY

1. Indian lotus fruits which had lain buried in a bed of peat probably for more than 200 years showed about 100 percent germination when the fruit coats were made permeable to water by a treatment with sulfuric acid.
2. These lotus fruits germinated well in 100 percent oxygen, 100 percent nitrogen, 100 percent hydrogen, and 100 percent carbon dioxid.
3. The length of sprouts from old fruits is always greater than that of sprouts from new ones.
4. Although they germinated thus in an external atmosphere containing no oxygen, an internal supply of oxygen was available to the embryo through the air contained in a cavity inside each fruit and in the intercellular spaces of the plant tissues. This gas contained all the oxygen necessary for the germination of the fruit.
5. The internal gas was pumped out of the fruit, collected, and analyzed. Each fruit contained about 0.2 cc. of gas. Seventy-nine analyses, when averaged, showed: (1) in the old fruits 18.33 percent oxygen, 0.74 percent carbon dioxid, and 80.93 percent nitrogen; (2) in fruits from the harvest of 1924, 18.88 percent oxygen, 0.81 percent carbon dioxid, and 80.31 percent nitrogen.
6. The respiration rate of air-dry fruits containing about 12 percent of water was so slow that a measurable amount of carbon dioxid was not obtained after three days' continuous absorption in sodium hydroxid.

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# A COMPARISON OF THE LIFE ACTIVITY OF CENTURY-OLD AND RECENTLY HARVESTED INDIAN LOTUS FRUITS<sup>1</sup>

ICHIRO OHGA

Contribution from Boyce Thompson Institute for Plant Research.

## INTRODUCTION

It was shown in a previous paper (5) that century-old and recently harvested fruits of Indian lotus differ in germination behavior, the old fruits giving more vigorous seedlings. The work here reported was undertaken to determine the relation of this fact to the rate of respiration, catalase activity, and hydrogen-ion concentration of the fruits.

## RESULTS

### Comparison of Catalase Activity of Dry Fruits

For the determination of catalase activity, a modified Appleman's (1) apparatus was used, and the amount of oxygen liberated from 10 cc. of hydrogen peroxid was used as a measure of the activity. This method was similar to that used by Crocker and Harrington (4). In preliminary tests, catalase determinations were made on fruit powder sifted through a 100-mesh screen, the amount used varying from 0.035 to 0.3 gram. A ten-minute reading was taken, and from this and from the weight of material used the catalase activity per gram was calculated. In all the experiments here reported, the activity was calculated in the same way. Table 1 shows the relation between the quantity of fruit powder used and the volume of oxygen liberated.

TABLE 1. *Catalase Activity of Lotus Fruits; Relation of Oxygen Liberated to Quantity of Fruit Tissue Used*

Weight in Grams	Cubic Centimeters of Oxygen Liberated							
	After 1 Minute		After 2 Minutes		After 5 Minutes		After 10 Minutes	
	Volume Observed (cc.)	Volume per Gram of Sample (cc.)	Volume Observed (cc.)	Volume per Gram of Sample (cc.)	Volume Observed (cc.)	Volume per Gram of Sample (cc.)	Volume Observed (cc.)	Volume per Gram of Sample (cc.)
0.30	21.0	70.0	35.7	119.0	51.2	172.0	54.5	181.0
0.15	8.5	56.5	14.5	96.5	27.6	184.0	37.3	249.0
0.07	4.5	64.5	9.0	129.0	17.5	250.0	24.1	345.0
0.035	2.2	63.0	4.3	123.0	8.1	231.0	11.7	335.0

<sup>1</sup> Published, at the expense of the Institute, out of the order determined by the date of receipt of the manuscript.

As shown in table 1, the maximum liberation of oxygen took place when 0.07 gram of fruit powder was used. Although the weight of half of a cotyledon (0.3 to 0.4 gram) exceeds this, the writer in practice used half of a cotyledon for each measurement. One plumule weighing approximately 0.025 gram was used in each test. Table 2 shows the results of the measurements of catalase activity of air-dry old and new fruits.

Table 2 shows that: (1) the catalase activity of the old fruits is much higher than that of the new fruits; and (2) catalase activity of the plumule is greater than that of the cotyledon.

TABLE 2. *Catalase Activity of Air-dry Old and New Lotus Fruits; Cubic Centimeters of Oxygen per Gram of Fruit Tissue*

Old Fruits		New Fruits	
Cotyledon	Plumule	Cotyledon	Plumule
208	357	172	344
224	280	152	200
221	332	167	304
306	363	159	410
256	362	181	300
243	339	166	311

### Comparison of Catalase Activity of Soaked Fruits

The fruits were soaked in tap water at 30° C., one lot of fruits being soaked for 12 hours and another lot for 5 days. Tables 3 and 4 show the catalase activity of old and new fruits after soaking in water.

TABLE 3. *Catalase Activity of Old and New Lotus Fruits Determined after 12 Hours' Soaking in Tap Water at 30° C.; Cubic Centimeters per Gram of Fruit Tissue*

Old Fruits		New Fruits	
Cotyledon	Plumule	Cotyledon	Plumule
123	105	109	94
118	119	108	106
120	123	114	110
120	116	110	107

TABLE 4. *Catalase Activity of Old and New Lotus Fruits Determined after 5 Days' Soaking in Tap Water at 30° C.; Cubic Centimeters per Gram of Fruit Tissue*

Old Fruits		New Fruits	
Cotyledon	Plumule	Cotyledon	Plumule
147	123	117	91
165	124	119	68
170	126	146	84
161	124	127	81

The results in tables 3 and 4 show that: (1) the catalase activity of old fruits is higher than that of new ones; (2) in soaked fruits the catalase activity of the cotyledon is greater than that of the plumule. This is the reverse of the condition found for air-dry fruits.

### Comparison of Catalase Activity in Anaerobically Treated Fruits

Series I: In order to determine the catalase activity after culture in oxygen-free condition, one lot was kept in cooled boiled water, and the other, after evacuation for ten minutes with a 400-mm. water-suction pump, was kept in cooled boiled water. In the latter case, sprouts did not develop. After four days the determinations of catalase activity were made. The results are shown in table 5.

TABLE 5. *Catalase Activity of Lotus Fruits after Being Left in Boiled Distilled Water for 4 Days; Cubic Centimeters of Oxygen per Gram of Fruit Tissue*

Treatment	Old Fruits		New Fruits	
	Cotyledon	Plumule	Cotyledon	Plumule
Coat on.....	172.0	60.2	91.3	47.2
	186.5	64.9	121.0	59.0
	182.4	66.2	112.4	52.7
	180.3	63.8	108.2	53.0
Coat stripped and evacuated with water pump..	88.0	33.0	43.6	17.0
	82.0	30.5	41.0	19.5
	92.2	29.5	50.7	16.7
	87.4	31.0	45.1	17.7

Series II: In order to determine the catalase activity in oxygen-free condition, the fruits, after the treatment above described, were kept in nitrogen gas. After four days the determinations were made. The results are shown in table 6.

TABLE 6. *Catalase Activity of Lotus Fruits Left in Nitrogen Gas for 4 Days; Cubic Centimeters of Oxygen per Gram of Fruit Tissue*

Treatment	Old Fruits		New Fruits	
	Cotyledon	Plumule	Cotyledon	Plumule
Coat on.....	142.2	59.1	104.3	41.5
	158.3	63.5	123.4	52.3
	134.5	67.2	116.7	47.8
	145.0	63.3	114.8	47.2
Coat stripped and evacuated with water pump..	82.5	38.0	67.0	33.9
	95.7	42.8	70.2	35.7
	103.5	45.2	59.8	31.6
	93.9	42.0	65.7	33.7



The results in tables 5 and 6 show that: (1) catalase activity is greater in old fruits than in new ones; and (2) catalase activity is greater in the cotyledon than in the plumule.

### Comparison of Respiration Rate in Dry and Wet State of New and Old Fruits

To determine the respiration rate during germination, a current of carbon dioxid-free air was drawn through a vessel containing the fruits and then through Pettenkofer tubes containing sodium hydroxid. The carbon dioxid absorbed was estimated by the double titration method of Brown and Escombe (3). Twenty fruits were used in each test. With fruits having intact coats a 3-day test did not show a measurable amount of carbon dioxid. But when 5 fruits with sprouts about 3 inches long that had been kept 3 days in a 30° C. incubator were transferred to a respiration chamber, much larger quantities of carbon dioxid were given off. The results are given in table 7.

TABLE 7. *Respiration of New and Old Fruits of Indian Lotus*

	Milligrams CO <sub>2</sub> Liberated	
	Old Fruits	New Fruits
Twenty air-dry fruits in 3 days at 25° C.....	0	0
Five soaked 1 day at 25° C.....	.012	.009
	.015	.005
	.015	.008
	.012	.007
	.015	.006
		.007
Average.....	.014	.007

The results in table 7 show that: (1) dry fruits after 3 days at 25° C. did not yield a detectable amount of carbon dioxid; (2) soaked fruits from which the plumule had just begun to emerge liberated measurable quantities of carbon dioxid; and (3) the respiration of old fruits was more intense than that of new ones.

### Comparison of Acidity in Dry and Wet State of Old and New Fruits

In order to determine the acidity of fruits, pH value was estimated by the quinhydrone-electrode method (2). Cotyledons and plumules were tested separately, using either a half of a cotyledon weighing approximately 0.35 gram in the dry and 0.75 gram in the wet condition, or an entire plumule weighing 0.025 gram in the dry and 0.10 gram in the wet condition. The plant tissue was ground in a mortar, 10 cc. of water were added, and the supernatant liquid was used for the determination. The results obtained are shown in tables 8 and 9.

TABLE 8. *Hydrogen-ion Concentration of Old and New Fruits of Indian Lotus; Determinations Made on Dry Fruits*

Old Fruits		New Fruits			
		Series I		Series II	
Cotyledon	Plumule	Cotyledon	Plumule	Cotyledon	Plumule
6.68	6.50	6.71	6.58	6.83	6.53
6.71	6.52	6.68	6.64	6.68	6.52
6.53	6.51	6.68	6.64	6.50	6.52
6.53		6.68	6.64	6.70	
6.56		6.68	6.64	6.70	
6.60	6.51	6.69	6.63	6.68	6.52

TABLE 9. *Hydrogen-ion Concentration of Old and New Fruits of Indian Lotus; Determinations Made on Soaked Fruits*

Old Fruits		New Fruits			
		Series I		Series II	
Cotyledon	Plumule	Cotyledon	Plumule	Cotyledon	Plumule
6.48	6.54	6.74	6.75	6.53	6.59
6.54	6.58	6.59	6.75	6.53	6.59
6.54	6.63	6.59	6.75	6.59	6.75
6.41	6.63	6.70	6.70	6.59	6.81
6.41	6.66				
6.46	6.64	6.65	6.74	6.56	6.69

Tables 8 and 9 show only small differences in the pH of tissues from old and new fruits. The error of a single determination by the method used was about 0.03 pH. The differences found were about two to four times this error, and in almost every comparison showed a slightly higher acidity in the old fruits than in new ones. It is not known whether this small difference, if it exists, is an important factor in contributing to the superior vigor of the seedlings of old fruits.

#### SUMMARY

1. The growth rate of old Indian lotus fruits was greater than that of new fruits.
2. The catalase activity of dry and soaked old fruits was greater than that of new ones.
3. The plumule showed greater catalase activity than the cotyledon in dry fruits, but in soaked fruits the reverse was true. The reason for this needs further investigation.
4. The respiration rate of dry fruits could not be measured because of

the slowness of respiration. However, when sprouted fruits were tested, it was found that the respiration rate of old fruits was greater than that of new ones.

5. Only small differences in the hydrogen-ion concentration were found, nearly all measurements, however, showing a higher acidity in the old fruits.

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## A DOUBLE MAXIMUM IN THE RATE OF ABSORPTION OF WATER BY INDIAN LOTUS SEEDS<sup>1</sup>

ICHIRO OHGA

Contributions from Boyce Thompson Institute for Plant Research.

### INTRODUCTION

In the course of a study of Indian lotus fruits (2), I had occasion to investigate the rate of water-absorption by the seeds. When the seeds were placed in water at various temperatures, the initial rate of water intake was rapid, this being followed by a decrease, then by an increase and a later decrease. This double maximum in the rate of water-intake appeared in the result of each of sixteen tests. The purpose of this paper is to describe and discuss the experiments which led to these results.

### METHODS

Five fruits of nearly similar shape, with smooth surface and perfect seed coats, were selected for each experiment. Each lot was placed in a small beaker filled with distilled water. The beakers were placed in incubators equipped with automatic temperature regulation. The temperatures selected were 5°, 20°, and 35° C. After having soaked for certain time intervals, the seeds were taken out, the surface water was wiped off with a dry cloth, and the seeds were weighed. The whole process of removing the seeds from the water and returning them to the same water again after they were weighed was accomplished in about a minute. The time required for weighing was not deducted from the total time interval.

At the start of each experiment the seeds were weighed every 15 minutes, later every 30 minutes, and finally every hour. Although the total possible absorption percentage is about 170 percent on the basis of air-dry weight, the experiment was discontinued when the absorbed water amounted to about 100 percent of the air-dry weight.

### RESULTS

Tables 1, 2, and 3 show the percentage of moisture-intake based on the air-dry weight. The figures in columns 3, 5, 7, 9, 11, and 13 are the differences in the absorbed percentage at every half-hour or every hour interval. Text figure 1 shows the differences in percentages at every hour interval, taking the time as abscissa and percentage of absorbed water as ordinate. As will be seen both in the tables and in the figure, the rate of moisture-intake by the seeds in the first 60 minutes was high, but in every case the hourly

<sup>1</sup> Published, at the expense of the Institute, out of the order determined by the date of receipt of the manuscript.

TABLE I. *Water Intake by Indian Lotus Seeds*  
(Temperature, 35° C.)

Time (Min- utes)	Experiment I		Experiment II		Experiment III		Experiment IV		Experiment V		Experiment VI		Ave. Percent Absorption	Ave. Rate per Half Hour
	Percent Absorption	Rate per Half Hour	Percent Absorption	Rate per Half Hour	Percent Absorption	Rate per Half Hour	Percent Absorption	Rate per Half Hour	Percent Absorption	Rate per Half Hour	Percent Absorption	Rate per Half Hour		
15	9.78*		9.89		9.04	9.42	8.74		8.82		8.82		9.28	
30	17.12	17.12	17.31	17.31	15.76	16.18	14.81	14.81	14.81	14.81	14.97	14.97	16.03	16.03
45	23.64		23.90		21.45	21.98	19.90		20.59		20.59		21.91	
60	29.35	12.23	29.95	12.64	26.87	11.11	24.76	24.76	9.95	9.95	28.88	9.90	27.18	11.15
75	34.24		35.16		30.49	32.13	28.88		8.01		32.62		31.63	
90	38.86	9.51	40.11	10.16	34.11	7.24	32.77	8.69	8.01		35.83	7.75	35.74	8.56
105	42.93		44.51		37.98		36.41		8.01		38.77		39.80	
120	47.28	9.42	48.90	8.79	42.38	8.27	40.78	6.52	10.92	10.92	42.51	6.15	43.77	8.03
135	54.08		54.12		47.29		45.89		10.92		46.79		48.25	
150	60.87	13.59	58.79	9.89	53.23	10.85	49.03	6.52	10.92		50.80	8.02	53.40	9.63
165	67.39		62.91		59.17		52.17		10.92		55.35		58.29	
180	73.10	12.23	67.03	8.24	64.60		54.83	5.80	10.92		62.03	8.56	62.92	9.52
195	79.08		71.43		70.02		58.45		11.41	11.41	67.01	12.56	68.16	10.34
210	84.78	11.68	75.19	8.52	75.19	11.59	74.03	7.25	10.92		73.26		73.26	
225	89.67		79.95		80.62		79.61		10.92		77.01	10.10	78.10	9.02
240	93.48	8.70	84.07	8.52	85.53	10.34	84.95	6.52	6.07	6.07	79.95		82.28	
255	96.74		87.36		89.41		88.35		6.07		82.62	5.61	85.51	6.08
270	99.46	5.98	90.39	6.32	93.02	7.49	91.02	5.07	4.85	4.85	84.76		88.36	
285	102.45		93.13		95.61		93.45				86.63	4.01	90.85	
300	105.16	5.70	95.88	5.49	98.45	5.43	95.87	4.83					92.83	4.47

\* Percentage of water absorbed, basis of air-dry weight.

TABLE 2. *Water Intake by Indian Lotus Seeds*  
(Temperature, 20° C.)

Time (Minutes)	Experiment I		Experiment II		Experiment III		Experiment IV		Experiment V		Ave. Percent Absorption	Ave. Rate per Hour
	Percent Absorption	Rate per Hour	Percent Absorption	Rate per Hour	Percent Absorption	Rate per Hour	Percent Absorption	Rate per Hour	Percent Absorption	Rate per Hour		
15	6.40*		6.67		6.61		6.58		6.93		6.64	
30	11.20		11.30		11.29		11.51		12.05		11.47	
45	16.00		15.07		15.43		15.89		15.96		15.67	
60	19.47	19.47	18.26	18.26	18.43	18.43	19.18	19.18	19.58	19.58	19.04	19.04
75	22.67		21.74		21.76		22.19		23.19		22.31	
90	25.60		24.93		24.79		25.21		26.58		25.47	
105	28.27		27.83		27.55		27.95		29.82		28.28	
120	30.93	11.46	30.43	12.17	30.03	11.60	30.41	11.23	32.53	12.95	30.87	12.83
135	33.33		32.76		32.23		32.60		34.94		33.17	
150	35.20		34.78		34.43		34.79		37.05		35.25	
165	37.07		36.52		36.36		36.99		39.16		37.22	
180	39.20	8.27	38.26	7.83	38.29	8.26	39.18	8.77	41.57	9.04	39.30	8.53
195	41.87		40.58		40.22		42.47		44.28		42.00	
210	45.07		43.48		42.42		46.58		47.89		45.09	
225	48.27		46.09		44.63		51.50		51.81		48.46	
240	50.93	11.73	48.41	10.15	46.84	8.55	54.79	15.61	55.12	13.55	51.22	11.92
255	53.87		50.72		48.76		57.53		58.43		53.86	
270	56.53		53.04		50.96		62.19		61.45		56.40	
285	59.20		55.36		53.17		60.00		64.16		58.82	
300	61.87	10.94	57.39	8.98	55.10	10.26	64.38	9.59	66.87	11.75	61.12	9.90
315	64.53		59.72		57.30		66.58		69.88		63.60	
330	67.47		62.32		58.95		68.22		72.89		65.97	
345	70.13		64.64		60.88		70.14		75.60		68.28	
360	72.80	10.93	66.66	9.27	62.53	7.43	71.78	7.40	78.31	11.44	70.42	9.30
390	77.87		71.59		66.94		75.62		83.43		75.09	
420	82.13	9.33	75.94	9.28	71.07	8.54	79.18	7.40	88.25	9.94	79.31	8.89
450	86.56		79.71		75.76		82.19		92.47		83.15	
480	88.80	6.67	83.19	7.25	80.44	9.37	85.21	6.03	96.39	8.14	86.81	7.50
510	92.00		86.67		84.04		87.95		99.70		90.07	
540	95.20	6.40	89.86	6.67	87.33	6.89	90.68	5.47	102.71	6.32	93.14	6.33
570	97.60		92.75		90.36		92.88		105.42		95.86	
600	99.47	4.27	95.07	5.21	93.11	5.78	95.07	4.39	107.83	5.12	98.11	4.97
630	101.34		97.89		95.32		97.27		110.24		100.31	
660	103.20	3.73	99.42	4.35	97.32	4.41	99.18	4.11	112.35	4.52	102.31	4.20
690	105.07		101.74		99.72		101.37		114.16		104.41	
720	106.40	3.20	103.77	4.35	101.65	4.13	103.29	4.11	115.66	3.31	106.15	3.84

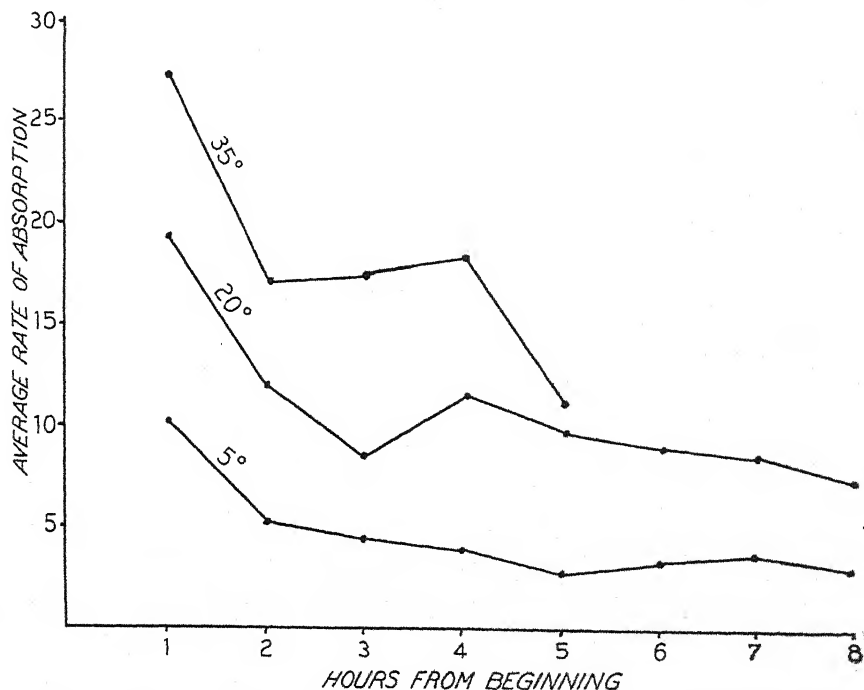
\* Percentage of water absorbed, basis of air-dry weight.

TABLE 3. *Water Intake by Indian Lotus Seeds*  
(Temperature, 5° C.)

Time (Minutes)	Experiment I		Experiment II		Experiment III		Experiment IV		Experiment V		Ave. Percent Absorption	Ave. Rate per Hour
	Percent Absorption	Rate per Hour	Percent Absorption	Rate per Hour	Percent Absorption	Rate per Hour	Percent Absorption	Rate per Hour	Percent Absorption	Rate per Hour		
15	3.11*		3.48		3.21		3.25		3.38		3.23	
30	5.37		6.22		5.61		6.00		6.23		5.83	
45	7.62		8.95		7.75		8.50		8.83		8.28	
60	9.32	9.32	10.94	10.94	9.89	9.89	10.75	10.75	11.16	11.16	10.41	10.41
75	11.01		12.43		11.23		12.25		12.47		11.82	
90	12.43		13.68		12.56		13.50		13.77		13.13	
105	13.84		14.92		13.63		14.75		15.06		14.39	
120	14.97	5.65	16.17	5.23	14.43	4.54	16.00	5.25	16.36	5.20	15.58	5.17
135	16.10		17.41		15.50		17.25		17.40		16.73	
150	17.23		18.65		16.37		18.25		18.44		17.82	
165	18.36		19.65		17.64		19.25		19.48		18.87	
180	19.20	4.23	20.64	4.47	18.71	4.28	20.00	4.00	20.32	4.16	19.81	4.23
210	21.46		22.38		20.58		22.50		22.60		21.91	
240	23.44	4.24	23.87	3.23	22.45	3.74	25.00	5.00	24.42	3.90	23.84	4.03
270	25.13		25.37		23.52		26.50		25.97		25.30	
300	26.55	3.11	26.86	2.99	24.32	1.87	28.00	3.00	27.28	2.86	26.60	2.76
330	28.24		28.10		25.39		29.75		28.57		28.01	
360	29.93	3.38	29.10	2.24	26.20	1.88	31.25	3.25	29.87	2.59	29.27	2.67
390	31.63		30.84		27.80		33.25		31.43		30.99	
420	33.04	3.11	32.58	3.48	29.40	3.20	35.00	3.75	32.99	3.12	32.60	3.33
450	34.74		34.07		30.47		36.25		34.55		34.07	
480	36.15	3.11	35.56	2.98	31.54	2.14	38.00	3.00	36.10	3.21	35.17	2.87
510	37.56		37.06		33.41		40.00		37.92		37.19	
540	38.70	2.55	38.55	2.99	35.01	3.47	41.75	3.75	39.48	3.38	38.70	3.23
570	40.10		39.80		36.09		43.25		40.78		40.00	
600	41.23	2.53	41.03	2.48	36.89	1.88	44.50	2.75	42.08	2.60	41.14	2.44
630	42.93		43.02		38.22		46.00		43.90		42.81	
660	44.62	3.39	45.01	3.98	39.56	2.67	47.50	3.00	45.45	3.37	44.43	3.29
690	46.03		46.51		40.36		48.75		46.49		45.63	
720	47.17	2.55	48.00	2.99	41.16	1.60	50.00	2.50	47.53	2.08	46.77	2.34

\* Percentage of water absorbed, basis of air-dry weight.

rate decreased to a first minimum, then increased to a second maximum, and then dropped to a final minimum. At 35° C., the half-hourly rate of absorption decreased from 16.03 to 8.03 and then rose to 10.34. At 20° C. the first minimum occurred after about 3 hours and the second maximum after about 4 hours. At 5° C., the differences in rate are not so marked as in the two former cases, but in each test a small secondary rise is shown.



TEXT FIG. 1. Average rates of absorption after various time intervals from the beginning. Ordinates are percentages on the basis of air-dry weight. Data taken from tables 1, 2, and 3.

The absorption rate gradually decreased after the second maximum of water-intake at about the time the plumule began to grow. The absorption diminished more quickly at higher temperatures. Thus, at 35° C. the plumule developed in 10 to 15 hours, at 20° C. in 20 to 26 hours, and at 5° C. it did not develop even after several weeks.

From the figure and the tables it can be seen that there appears a change in the rate of moisture-intake at about 50 percent water-absorption after 2 hours at 35° C., at about 40 percent after 3 hours at 20° C., and at about 30 percent after 36 hours at 5° C.

#### Experiments Relating to the Cause of the Double Maximum

*Hydrogen-ion Concentration.* Since it has been found by MacDougal (1) that the swelling of colloid material is influenced by the hydrogen-ion



concentration, measurements were made of the pH value of extracts of the seeds after different periods of soaking. Only small changes in pH were found, and these did not appear to be related in any way to the change in rate of absorption.

*Air Pocket.* There is a small air pocket in the center of each seed. In order to determine whether the second maximum in water intake is related to the infiltration of water into this pocket, the rate of absorption of cotyledons only was measured. The results are shown in table 4. The double maximum is shown in the results at 35° and 20° C., the secondary rise at 5° C., however, being small. The presence of an air pocket is not necessary for a second maximum in rate of water-intake.

TABLE 4. *Water Intake by Cotyledons of Indian Lotus Seeds*

Time (Hours)	Temperature, 5° C.			Temperature, 20° C.			Temperature, 35° C.		
	Experi- ment I	Experi- ment II	Aver- age	Experi- ment I	Experi- ment II	Aver- age	Experi- ment I	Experi- ment II	Aver- age
1	23.25	22.97	23.11	33.04	35.20	34.12	34.60	33.1	33.9
2	9.80	10.81	10.31	12.46	12.72	12.59	19.2	21.4	20.3
3	8.96	9.46	9.23	11.90	11.84	11.87	23.6	25.4	24.5
4	6.50	7.84	7.17	12.16	12.42	12.29	16.7	16.8	17.1
5	6.45	6.21	6.33	11.59	13.32	12.46	12.3	8.6	10.1
6	7.84	6.49	7.17	9.28	9.76	9.52	6.0	5.8	5.7
7	6.72	5.67	6.20	9.57	8.88	9.23	5.3	4.1	4.8
8	5.89	7.03	6.46	6.37	6.84	6.61			
9	5.60	5.41	5.51	5.22	3.55	4.39			

### Double Maximum in Shull's Results with Split Pea

When *Xanthium* seeds were soaked, no double maximum was found; hence it appeared possible that the change in rate with lotus seeds was

TABLE 5. *Water-absorption by Seeds of Split Pea from Data of Shull (3)*

Time (Minutes)	Variety of Split Pea		Green Canada Field Pea		Small Scotch Yellow		Tom Thumb Yellow	
	H <sub>2</sub> O Intake	Half-hour Rate	H <sub>2</sub> O Intake	Half-hour Rate	H <sub>2</sub> O Intake	Half-hour Rate	H <sub>2</sub> O Intake	Half-hour Rate
1	4.25		4.09		5.38		3.76	
15	16.20		16.68		25.09		15.50	
30	23.84	23.84	22.98	22.98	33.69	33.69	21.15	21.69
45	30.14		28.78				26.16	
60	35.32	11.48	34.96	11.98	50.89	17.20	30.11	8.96
75	40.30		42.52		58.24		33.33	
90	45.22	9.90	50.41	16.45	69.59	18.70	37.28	7.17
105	52.06		64.76		68.10		45.34	
120	57.50	12.28	73.96	23.55	70.34	.75	51.61	14.33
135	61.42		79.48		72.58		57.21	
150	65.34	7.84	82.99	9.03			62.01	11.40
165	68.60						67.16	
180	70.32	4.98					70.74	10.73
195	72.18							
210	72.97	2.65						

related to the thickness of the cotyledons. Since Shull (3) in 1920 had measured the rate of intake of water by split pea seeds, which also have thick cotyledons, a recalculation of his data was made in order to show the half-hourly amounts of absorption. When this was done, a double maximum was found in Shull's results with split pea (table 5).

Although Shull did not use the words "double maximum" in this case, he noted this change in rate and calls attention to it in the following words (3, page 380): "After a certain critical percentage has been reached, however, they show a remarkable rise above the ideal curve indicated by the first part of the absorption."

In table 5, which is taken from Shull's paper, the half-hourly rates are shown in columns 3, 5, 7, and 9. The unknown variety of split pea shows a first minimum at about 90 minutes and a second maximum at about 120 minutes. Green Canada field pea and Tom Thumb Yellow also show second maxima, but the result with Small Scotch Yellow is doubtful.

#### SUMMARY

1. When Indian lotus seeds were soaked in water at different temperatures, there appeared a double maximum in the rate of water-intake.

2. At 35° C., the first minimum in rate occurred after about 120 minutes' soaking, and the second maximum after about 210 minutes; at 20° C. these changes in rate occurred after 180 minutes and 240 minutes, respectively; at 5° C. the differences, although less marked quantitatively, appeared after about 300 minutes and 420 minutes.

3. The changes in rate of water-absorption were not found to be correlated with change in hydrogen-ion concentration.

4. The separated cotyledons also showed a second maximum in rate of water-intake, indicating that infiltration of the internal air pocket was not a factor.

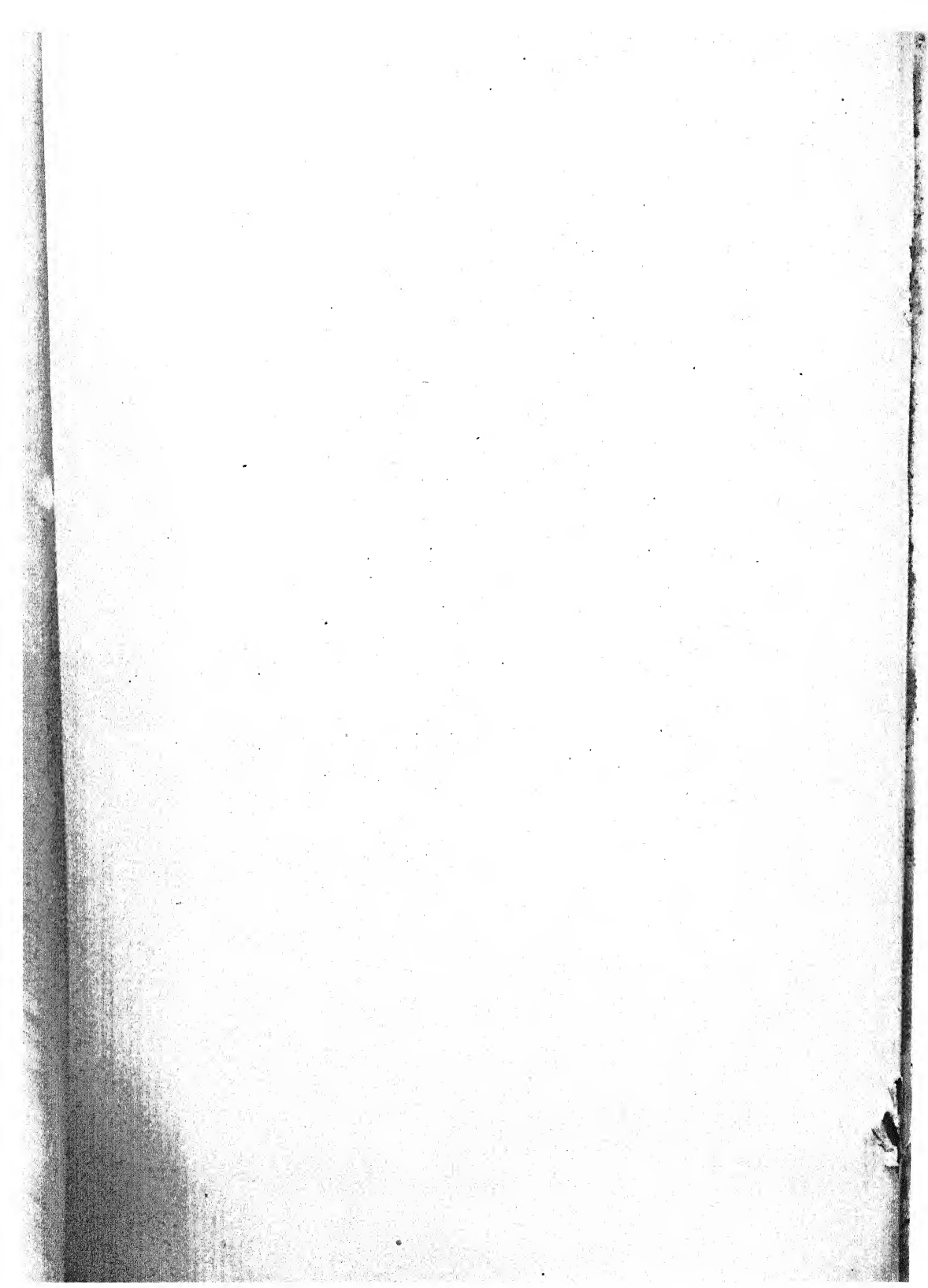
5. A recalculation of the data obtained by Shull (3) in 1920 with split peas showed second maxima in rates of water-absorption. Shull also noted this change in rate and calls attention to it in the paper cited.

6. The cause of the appearance of the second maximum is not clear. It is probable, however, that it is related to the nature and structure of the seed materials, especially to their thickness.

EDUCATIONAL INSTITUTE,  
DAIREN, MANCHURIA

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## ACIDIFICATION OF UNBUFFERED SALT SOLUTIONS BY PLANT TISSUE, IN RELATION TO THE QUESTION OF TISSUE ISOELECTRIC POINTS

F. E. DENNY AND W. J. YOUNG

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### INTRODUCTION

In all cases observed by us, when plant tissue is placed in contact with a tenth-normal solution of calcium chlorid the solution soon becomes acidified. Within an hour, under suitable experimental conditions, the hydrogen-ion concentration may increase ten, twenty, or thirty fold. What is the cause of this development of acidity? Should this effect be interpreted as evidence that the tissue is absorbing cations faster than anions? And if so, is the process of ion absorption related to the amphoteric character of the proteins in the plant? This problem is important because it is related to the question of whether plant tissue in absorption of ions behaves like an ampholyte with an isoelectric point. Through this connection we first became interested in it.

The effect of seeds upon the hydrogen-ion concentration of salt solutions was studied in detail by Rudolphs (6, 7, 8), who soaked the seeds of different species in various salt solutions of different concentrations, and then determined the pH of the solution surrounding the seeds. He was impressed by the tendency of the seeds to bring the pH of the various salt solutions to definite values, each final value being fairly constant for a given species of seed regardless of the original pH or concentration of the salt solution (except in the case of very dilute solutions). Thus the final value for corn (*Zea mays*) was between pH 3.9 and 4.2 when the seeds were soaked in such varied chemical solutions as  $K_2SO_4$ ,  $KNO_3$ ,  $CaCl_2$ ,  $H_2SO_4$ ,  $HCl$ ,  $KCl$ ,  $BaCl_2$ , oxalic acid, citric acid, etc. Rudolphs' conclusion was that "the chemical properties of the chief protein constituent of the seeds seem responsible for the changes in hydrogen-ion concentration." Scott (9) found that the mycelium of *Fusarium lycopersici* was able to change the reaction of several different dilute salt solutions to pH 5.4 which was regarded as the isoelectric point of the tissue. Thus, the behavior of the living mycelium was thought to be analogous to that of an amphoteric colloid.

We have experimented upon the effect of plant tissue upon the pH of unbuffered salt solutions, using 6 different tissues, 15 different salt solutions, with 3 to 5 different concentrations of each salt. In our experiments we

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did not find that tissues were able to bring the pH of the solutions to characteristic values. The values obtained for any given tissue varied with the salt used and especially with the nature of the cation and the concentration of the salt. It is true that when a series of dilutions of a salt solution was made a characteristic value was approached, but this value was merely the pH of the water-extract of the tissue made under the same conditions.

In these experiments with unbuffered salt solutions the materials which leached out of the tissue into the salt solution were important factors in establishing the pH of the external solution. The situation is analogous to that obtained in our previous experiments (12) with buffered salts, with this important difference: the substances leaching out of the tissue reacted with certain cations in such a manner as to produce a hydrogen-ion concentration greater than either that of the water-extract of the tissue or of the salt solution used.

Acidifying effects similar to those shown by the tissue in contact with a salt solution were also obtained by the addition of the appropriate salt to that portion of the water-extract of the tissue which passed through collodion in dialysis, showing that non-colloidal substances were important. Finally, we were able to obtain analogous results by adding salts to mixtures of organic acids such as malic, succinic and aspartic, and to solutions of phosphates and pectin.

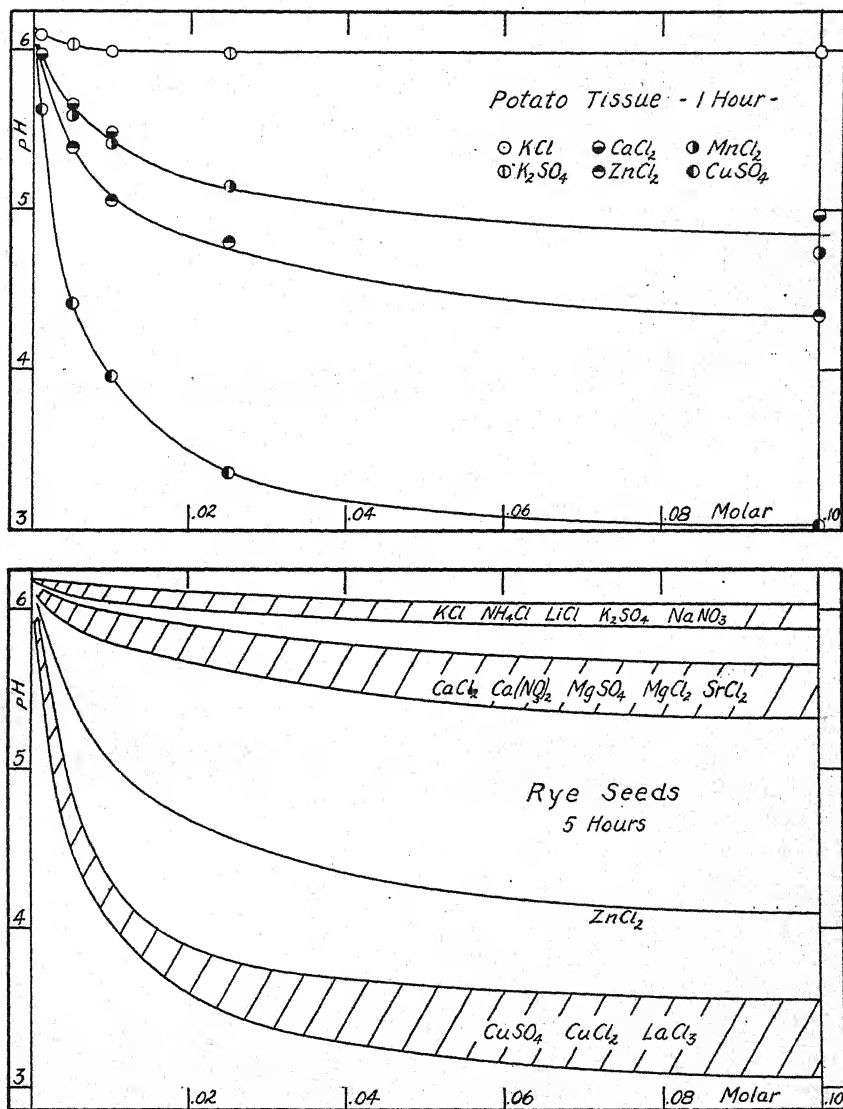
Although our results do not show that the tissues themselves or the proteins in the tissue take no part whatever in the observed changes in pH, they indicate that the interpretation of these effects as being due to the amphoteric character of the proteins, or as indicating the existence of an isoelectric point for the tissue or protoplasm, was far too sweeping.

## EXPERIMENTAL RESULTS

### The Effect of Plant Tissues upon the pH of Salt Solutions

In this series of experiments the plant tissue was placed in large test tubes containing the salt solution, the weights of tissue and the volumes of the salt solution being shown in table 1. The test tubes containing the mixture of tissue and salt solutions were then rotated end-for-end on a turning bar. At intervals a sample of the liquid was removed and the pH was determined by the quinhydrone method (1). Table 1 shows the results obtained by a representative experiment and the results of other experiments are shown in text figure 1.

The results in table 1 and text figure 1 show that the final pH value with these tissues depended upon the salt used and upon the concentration of the salt. Monovalent cations like potassium had much less effect than divalent cations like calcium or magnesium. The effect of a given salt in producing acidification decreased as the concentration decreased, but this effect was unmistakable even in concentrations of 0.001M in the case of copper sulphate, and in most cases also with 0.001M calcium chlorid.



TEXT FIG. 1. Ordinates show the pH values of the external solution when 25 grams of tissue were immersed in 25 cc. of various salt solutions of the concentrations shown as abscissae. In the graph for rye seeds the salts listed in the shaded areas gave similar results, all the data for these salts falling within the limits shown. Note that each tissue did not bring the external solution to definite pH values, but that the values obtained varied with the salt used, with the concentration of the salt, and especially with the nature of the cation.

Potassium salts produced only slight acidifications except with whole corn seeds. It appears that a factor is operating in the case of whole corn seeds that is less important with the other tissues studied. This point is discussed in a later paragraph.

The magnitude of the acidifications produced may be estimated in the case of corn-seed powder by comparing the pH of the water-extract, 6.19, with that obtained by bringing the seed powder in contact with 0.1M  $\text{CaCl}_2$ , which after one hour was found to be 4.68. Since pH 6.19 represents a hydrogen-ion concentration of  $6.5 \times 10^{-7}$  and since pH 4.68 represents a hydrogen-ion concentration of  $210 \times 10^{-7}$ , it is seen that the hydrogen-ion concentration has been increased about 30-fold by the presence of the calcium chlorid.

We did not find it feasible to obtain what may be called a "final equilibrium." One experiment was continued for twenty-two and one-half hours but certain tissues showed evidences of fermentation and it was concluded that the results for longer periods than nine hours were not to be depended upon. For extended periods of observation protection against the action of micro-organisms should be provided, but in such cases it would be necessary to take into consideration the effect of the preservative upon the process studied. At any rate, at whatever period the experiment was stopped the observed pH was different in the different salt solutions, and in different concentrations of the same salt.

In this connection it is necessary to consider the question to what extent the acidities developed with the different salts were related to the pH values of the salt solutions themselves. The KCl solutions were neutral in reaction, and the  $\text{CaCl}_2$  solutions were nearly so, the pH values varying from about 6.5 to 7.0. The copper sulphate solutions were acid in reaction, however, the pH values being 4.9, 5.2 and 5.5 for concentrations of 0.1, 0.01, and 0.001M respectively; but it will be noted in text figure 1 that the acidities developed upon adding the tissues were much greater than could be accounted for upon the basis of the acidities of the copper sulphate solutions themselves.

The essential points in connection with the data in table 1 and text figure 1 are that (1) we find no definite pH value which is constant and characteristic for a given species in different salt solutions; there is no evidence that the observed results are due to the presence of proteins or other amphoteric colloids with definite isoelectric points; (2) it would be impossible to find a definite pH value as an isoelectric point for a tissue by bringing the tissue in contact with various salt solutions, unless for this purpose very dilute solutions were used; and the extent to which the dilution of the salt would have to be carried in order to reach a definite pH value would vary with the salt used, *e.g.*, approximately 0.01M for KCl, 0.001M for  $\text{CaCl}_2$ , and even more dilute for  $\text{CuSO}_4$ . Under such conditions, of course, the end-point toward which the values approach upon dilution



TABLE 1. *Acidity of the External Solution after adding Plant Tissue to Salt Solutions*

Salt Used	M Conc.	pH Values of External Solution											
		Potato After 1 hr.	Apple After 1 hr.	Carrot After:		Corn, Whole Seeds After:		Corn, Seed Powder After:		Rye, Whole Seeds After:		Wheat, Whole Seeds After:	
				1.5 hrs.	9 hrs.	1 hr.	9 hrs.	1 hr.	2 hrs.	1 hr.	9 hrs.	1 hr.	5 hrs.
None....	H <sub>2</sub> O, Check	6.12	3.54	6.47	5.95	5.76	5.21	6.20	6.20	6.42	6.14	6.93	6.73
" ....	"	6.12	3.50	6.49	5.95	5.85	5.37	6.22	6.18	6.42	6.14	6.86	—
" ....	"	6.09	—	—	—	6.09	5.44	—	—	6.43	6.05	—	—
KCl ....	0.1	5.90	3.54	6.34	5.90	4.26	4.31	6.00	6.03	6.17	6.02	6.60	6.50
" ....	0.01	6.07	3.48	6.51	5.96	4.93	4.73	6.13	6.10	6.32	6.07	6.81	6.67
" ....	0.001	6.10	3.48	—	—	5.94	5.21	6.15	6.12	6.41	6.22	6.88	6.72
CaCl <sub>2</sub> ..	0.1	5.22	3.16	5.78	5.51	3.87	3.92	4.68	4.68	4.02	4.34	5.71	5.71
" ....	0.01	5.54	3.39	6.22	5.84	4.09	4.09	5.40	5.40	5.59	5.37	6.32	6.36
" ....	0.001	6.02	3.49	6.41	5.97	4.93	4.73	6.07	5.97	5.93	5.70	6.87	6.71
CuSO <sub>4</sub> ..	0.1	2.99	2.62	—	—	3.34	3.29	3.24	3.28	3.63	3.55	3.89	3.82
" ....	0.01	4.00	2.99	4.73	5.27	3.75	3.70	4.92	4.97	4.02	3.95	4.50	4.60
" ....	0.001	5.61	3.46	4.73	5.24	4.92	4.56	5.98	5.99	5.43	5.54	6.43	6.42

Note: 25 grams of tissue + 25 cc. of salt solution, except corn seed powder in which case 50 cc. of salt solution was used. Potato, apple and carrot tissue consisted of disks 1 cm. in diameter x 1 mm. in thickness.

is the pH of the solution obtained by bringing the tissue in contact with water instead of salt solution.

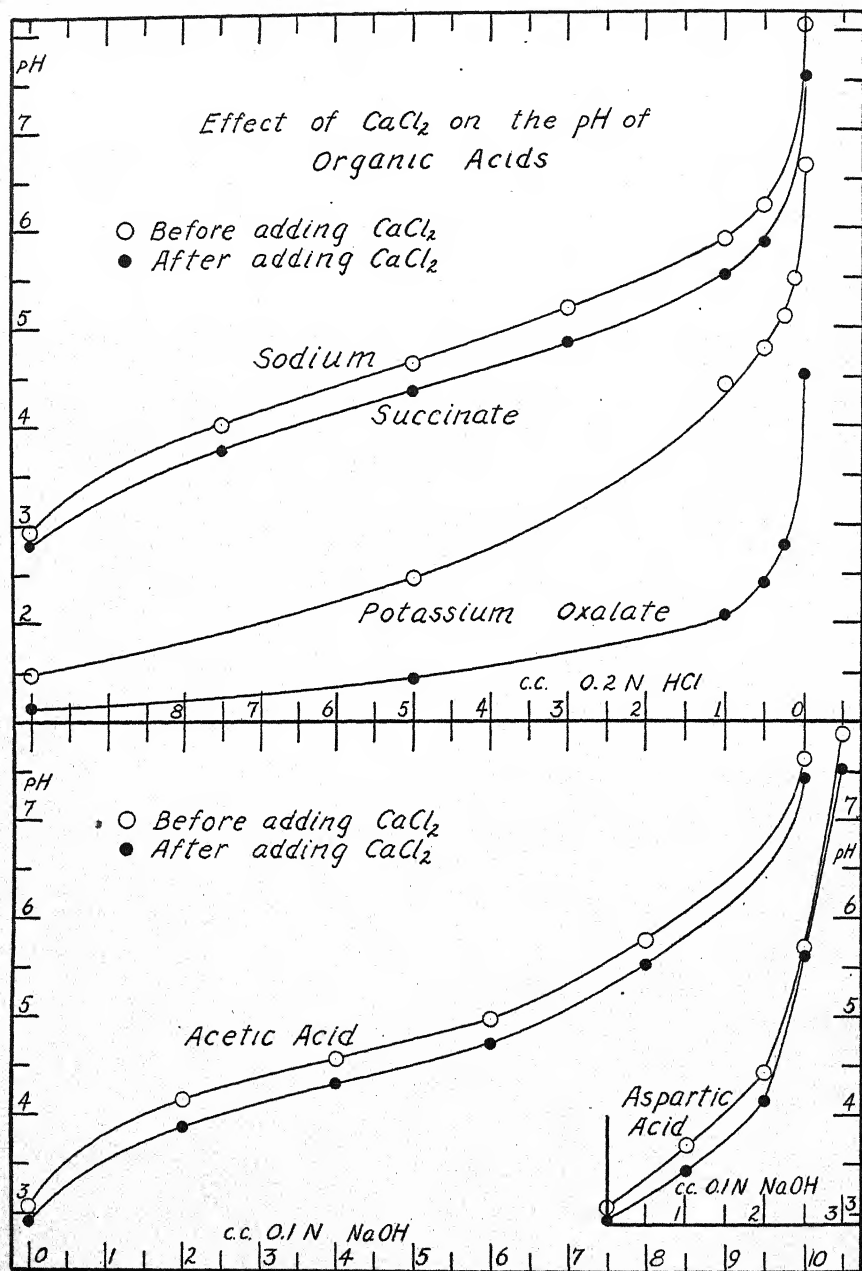
### The Effect of Adding Salts to the Water-extracts of the Tissues

The data in table 2 show that to obtain acidification effects with salts it is unnecessary to add the salt solutions to the tissue itself. We may add an equal amount of salt to the water-extract of the tissue and obtain results similar, at least qualitatively, to those obtained by bringing the salt solution in contact with the tissue.

TABLE 2. *Acidity Developed by Adding Salts to the Water-extracts of Tissues*

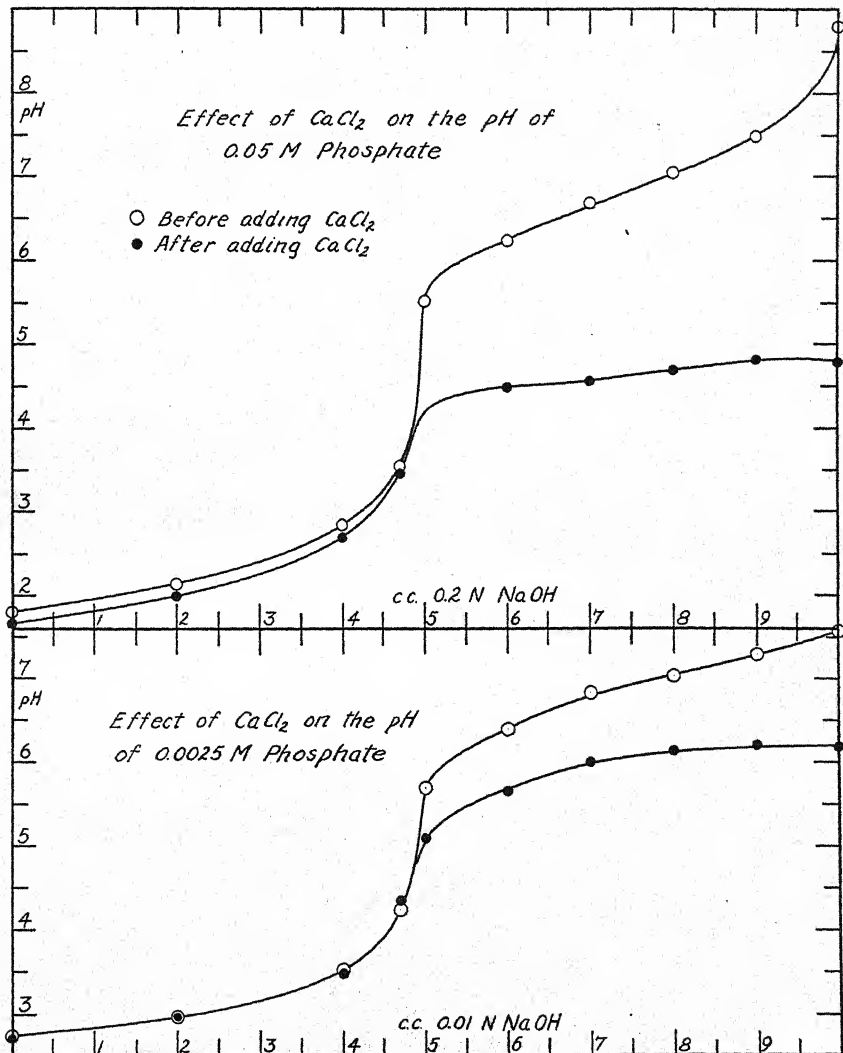
Salt Added	M Conc.	Potato Extract	Apple Extract	Carrot Extract		Corn, Whole Seed Extract	Corn, Seed Powder Extract	Wheat, Whole Seed Extract
				After 1.5 hrs.	After 9 hrs.			
None. . .	—	6.21	3.54	6.46	6.24	5.74	6.27	7.12
" ....	—	—	3.54	—	—	5.66	—	—
KCl ....	0.1	5.93	3.45	6.42	6.22	5.50	6.16	6.84
" ....	0.01	6.14	3.50	6.54	6.19	5.90	6.22	7.01
" ....	0.001	6.17	3.51	—	—	5.73	6.24	7.06
CaCl <sub>2</sub> ..	0.1	5.24	3.05	6.04	5.76	5.15	4.22	6.02
" ....	0.01	5.41	3.37	6.39	6.07	5.34	5.00	6.60
" ....	0.001	5.98	3.47	6.58	6.20	5.54	6.11	6.96
CuSO <sub>4</sub> ..	0.1	2.92	2.53	—	—	3.13	2.89	3.89
" ....	0.01	3.61	2.91	3.68	3.67	3.77	3.67	4.30
" ....	0.001	5.34	3.34	5.15	5.19	4.39	5.90	5.96





TEXT FIG. 2. Acidifications obtained by adding  $\text{CaCl}_2$  to organic acid solutions previously adjusted to various pH values by adding the amounts of acid or alkali shown as abscissae. Curves with circles show pH values before adding  $\text{CaCl}_2$ , curves with black dots show the pH values after adding  $\text{CaCl}_2$ . Precipitation occurred in the case of potassium oxalate.

The experimental procedure was as follows: The amounts of tissues were the same as for table 1; to each lot water was added instead of the salt solution; after thorough mixing on the turning-bar for the time periods shown in table 1, the water-extract was poured off, and centrifuged or filtered or both; the appropriate salt was then added to each lot, the amount of salt added to the water-extract being equal to the amount of salt in the solution added to the tissue.



TEXT FIG. 3. Acidifications produced by adding  $\text{CaCl}_2$  to phosphoric acid solutions previously adjusted to various pH values by adding the amounts of alkali shown as abscissae. Curves with circles show the pH values before adding  $\text{CaCl}_2$  and the black dots show the pH values after adding  $\text{CaCl}_2$ . Note that acidifications were produced at pH values higher than about 4 to 5.

Again we find surprisingly large effects upon the pH value; thus, in the case of the potato, the addition of  $\text{CaCl}_2$  to the water-extract in amounts sufficient to make the solution  $0.1M$  with respect to  $\text{CaCl}_2$  changed the pH from 6.21 to 5.24. This represents a change in hydrogen-ion concentration from  $6.2 \times 10^{-7}$  to  $58 \times 10^{-7}$ , *i.e.*, the  $\text{H}^+$  has increased about nine-fold. In the case of the water-extract of the corn seed-powder the change was from  $5.4 \times 10^{-7}$  to  $600 \times 10^{-7}$ , *i.e.*, the  $\text{H}^+$  after adding the  $\text{CaCl}_2$  was more than 100 times that before adding it.

Acidifications similar to those obtained by bringing the salts in contact with the tissues were obtained with all extracts except in the case of the water-extract of whole corn seeds, which did not give as much increase in hydrogen-ion concentration upon adding  $\text{KCl}$  and  $\text{CaCl}_2$  as was obtained by adding the salt solutions to the whole seeds. Only small amounts of material leached out of the corn seeds and the water-extract was consequently poorly buffered. It may be that in this case the influence of the substances which leach out of this tissue was of less importance, and that adsorption on the surface of the seeds was the principal factor.

We verified the observation of Rudolphs (7) that the outer seed coat layer when stripped from the corn seed and placed in salt solutions causes an increase in the hydrogen-ion concentration of the solution. But this is not due to the protein in the seed coat since, according to the results of microchemical tests, protein is not present in these coat layers or is present only in traces. The acidification of calcium chlorid solutions of  $0.1$  and  $0.01M$  concentration was found to occur even when the seed coats had been thoroughly extracted with alcohol, ether, and water before being placed in the salt solution. That copper was taken up by the seed coats was shown by the following experiment. The coats after separation from the seeds and after extraction by alcohol, ether and water were placed in a  $0.1M$  solution of copper sulphate; the coats were then removed and rinsed with water until the rinse-water gave no further test for copper with potassium ferrocyanid; the coats were then extracted with dilute acetic acid and this extract gave a test for copper; ferrocyanid tests upon the seed coats showed that copper had been taken up by them.

No doubt the adsorption factor was operative not only in the case of whole corn seed but also in the cases of the other tissues. But since these other tissues gave a more highly buffered water-extract with larger amounts of substances for reaction with the salts it seems likely that the adsorption effect was of secondary importance in producing acidification.

### **Effect of Boiling and Dialyzing the Water-extracts upon their Capacity to Produce Acidification**

Since it was found that the water-extract of the tissue could produce effects comparable to those produced by the tissue itself, the question arose as to whether the effective constituents in the water-extract are

soluble proteins. This point was determined by boiling the water-extract and also by dialyzing it in collodion bags; the first method eliminated the proteins coagulable by heat, and the second eliminated not only all proteins, but also all colloidal matter. In the process of dialysis the materials passing through collodion were recovered and were evaporated back to somewhat less than the original volume, so that when the dialysis was completed the water-extract had been separated into two fractions, one representing the substances passing through collodion, and the other the substances held back by it.

Table 3 shows the results of experiments with the boiled and dialyzed water-extracts in producing acidification upon the addition of salts. Column 4, table 3, shows the effects produced by the tissue itself, and the other columns show the effect of adding salts to the water-extract before boiling, after boiling, and after dialysis.

TABLE 3. *Comparison of Effectiveness of Tissues and of Extracts of Tissues in Producing Acidification on Addition of Salts*

Tissue Used	Salt Added	M Conc.	pH Observed After Salts Were Added			
			To Tissue	To a Water Extract of Tissue		
				Before Boiling	After Boiling	After Dialysis
Apple	None	H <sub>2</sub> O, Check	3.59	3.59	3.66	3.90
	KCl	0.1	—	3.56	3.65	3.87
		0.01	3.56	3.60	3.65	3.82
		0.001	3.52	3.55	3.65	3.80
	CaCl <sub>2</sub>	0.1	3.27	3.13	3.36	3.55
		0.01	3.47	3.44	3.55	3.80
		0.001	3.51	3.51	3.65	3.89
	CuSO <sub>4</sub>	0.1	2.78	2.67	2.85	2.97
		0.01	3.10	3.02	3.16	3.34
		0.001	3.51	3.43	3.52	3.75
Corn Seed Powder	None	H <sub>2</sub> O, Check	6.42	6.42	—	—
	KCl	0.1	6.40	6.40	6.47	6.00
		0.01	6.28	6.31	6.41	5.87
		0.001	6.41	6.39	6.47	5.96
	CaCl <sub>2</sub>	0.1	6.41	6.40	6.47	5.96
		0.01	4.88	4.49	4.53	4.61
		0.001	5.87	5.48	5.20	5.20
	CuSO <sub>4</sub>	0.1	6.35	6.26	6.27	5.85
		0.01	3.53	3.11	—	3.29
		0.001	5.36	4.87	—	3.95
	CuCl <sub>2</sub>	0.1	6.26	6.03	—	5.43
		0.01	3.34	2.94	2.97	3.03
		0.001	5.53	4.89	4.16	3.89
		0.001	6.29	6.07	6.01	5.42

Care was taken to keep the volume and concentration relationships comparable; the same amount of salt was added to the extracts as was added to the tissue; the volume of the water-extract taken as a sample in

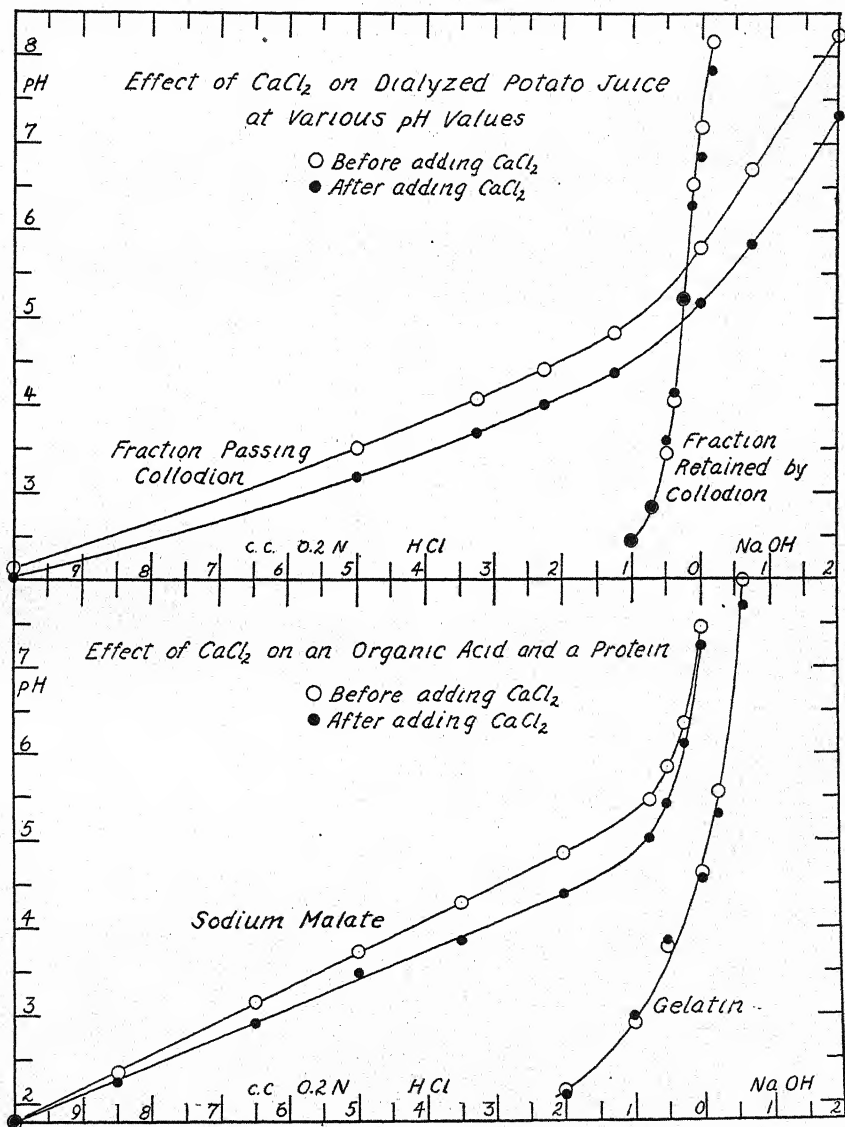
each case represented the soluble matter from the same number of grams of tissue as that to which the salt solution was added; after boiling and dialysis, the volumes were adjusted in such a manner that after the addition of the salt solution the original volume would be obtained. For example, the procedure for the apple tissue was as follows: 25 grams of apple disks were placed in 25 cc. of the solutions shown in columns 2 and 3, table 3, in large test tubes; after rotation end-for-end on a turning-bar the solutions were drained off into a measuring cylinder; the tissue that remained in the test tubes was rinsed<sup>2</sup> with small amounts of water, the rinsings being added to the liquid in the cylinder until the volume was 50 cc. The pH values of the lots in the different salt solutions are shown in column 4, table 3. The water-extract was obtained by adding 25 cc. of water to 25 grams of tissue in a number of small bottles, rotating these, decanting into a measuring cylinder, rinsing the tissue with small quantities of water, adding the rinsings to the liquid in the cylinder; to an aliquot of this solution equivalent to the number of grams of tissue used in column 1, table 3, the correct number of cc. of salt solution (*e.g.* using 1.0 *M*  $\text{CaCl}_2$  in preparing the 0.1 *M*  $\text{CaCl}_2$  lot) were then added, and the volume finally adjusted to equal the volume of liquid when the salt solutions were added to the tissues. The pH values of this series were obtained and are shown in column 5, table 3. The data for column 6, table 3, were obtained with the boiled water-extracts. A portion of the water-extract was boiled for five minutes, cooled to room temperature, and centrifuged and filtered. The volume was then adjusted to the amount originally taken and, to an aliquot of this solution equivalent to the amounts of tissue used in column 4, salts were added to give the same concentrations of salts as were added to the tissue. The pH values that were obtained are shown in column 6, table 3. The data for column 7, table 3, were obtained by adding the salts to the fraction of the water-extract that passed through collodion during dialysis, after adjusting the volume of this fraction by evaporation so that comparable volume relationships were maintained.

We tested each collodion bag for imperfections at the end of the dialysis period by placing a congo-red solution inside the bag and noting whether any congo-red passed through. In the cases of the potato, apple, corn seed powder, and wheat seed powder water-extracts, the portion passing through the collodion was tested for protein by adding acetic acid and sodium acetate to give 0.01 *N* concentrations of each, and heating on a boiling-water bath for one-half hour. No precipitates were formed in any of the dialyzed extracts as a result of this test.

Table 3 shows that the acidification that resulted from adding salts to the tissue was also obtained by adding salts to the water-extract both before and after boiling, and even to the fraction that passed through collodion during dialysis. An attempt was made to determine the behavior of the

<sup>2</sup> In this respect the procedure differs from that in tables 1 and 2, in which the liquids were obtained by decanting from the tissue without subsequent rinsing.

fraction held back by collodion when salts were added. It was found, however, that this fraction was so poorly buffered that it was not practicable to make hydrogen-ion measurements with it. Adding salts to the non-dialyzable fraction produced the same effect as adding the same salts to



TEXT FIG. 4. Upper half of figure compares the acidifications produced by adding  $\text{CaCl}_2$  to the fraction of potato juice which passed through collodion with those obtained by adding  $\text{CaCl}_2$  to the non-dialyzable portion. Lower half shows that acidifications were produced by adding  $\text{CaCl}_2$  to sodium malate solution but not by adding  $\text{CaCl}_2$  to gelatin solution. Results with dialyzed fractions were analogous to those obtained with tissues, but results with protein or protein-containing solutions were not.



water. Further evidence on this point is shown by text figure 4 which indicates the effect produced by adding  $\text{CaCl}_2$  to the non-dialyzable fraction, not of the water-extract of potato, but of potato juice. Although the non-dialyzable fraction of potato juice had more solids in it than the non-dialyzable fraction of the water-extract of potato disks, it is seen that the addition of  $\text{CaCl}_2$  had no important effect upon the pH.

Table 3 shows that the acidification of salt solutions by the tissues should not be interpreted as being due merely to reactions of salts with proteins or other colloidal materials. The soluble substances, non-colloidal and non-protein, which diffuse out of the tissue into the salt solution are important factors in producing this acidification by reacting with the salts.

### Acidification of Organic Acid Solutions by the Addition of Salts

We have shown that the soluble substances which leach out of the tissue into the salt solution are important factors in causing the acidification that is observed when the tissue is placed in contact with the salt solution. Two questions may now be taken up: What substances are responsible for this effect? How do they cause increased hydrogen-ion concentrations?

The results of our experiments indicate that organic acids may be important factors, since acidifications similar qualitatively to those given by adding salts to water-extracts of tissues can be obtained by adding salts to organic acids or mixtures of organic acids with their salts. The curves in text figure 2 show the effects upon the pH of different organic acid solutions resulting from the addition of calcium chlorid. Solutions of the organic acids were made up and adjusted to various pH values by the addition of acid or alkali. The abscissae of each curve show the amount of acid or alkali added to 10 cc. of each organic acid solution; water was then added to give a final volume of 20 cc.; this solution was thoroughly mixed and divided into two equal parts, to one of which 1 cc. of 1.0  $M$   $\text{CaCl}_2$  solution was added and to the other 1 cc. of  $\text{H}_2\text{O}$ . The pH values that were obtained were plotted as ordinates in each curve of text figure 2.

All the organic acids tested showed an acidifying effect upon the addition of  $\text{CaCl}_2$ . This increase in hydrogen-ion concentration upon the addition of  $\text{CaCl}_2$  occurred at all points on the titration curve, at least up to pH 7.5, except in the case of aspartic acid which did not show acidifications at pH values more alkaline than about 4.5. Asparagine showed such poor buffer action that pH measurements were uncertain; however, small increases in hydrogen-ion concentration were observable.

It is important in this connection to determine whether these acidifications occur when low concentrations are used and when the pH is adjusted to values that are characteristic of plant juices or water-extracts of plant tissues. To test this point salts were added to two different solutions of organic acids as follows: (1) A solution of malic acid and sodium malate made by adding 10.4 g. of malic acid to a solution containing 5.2 g. of malic

acid that had been neutralized with sodium hydroxid and adjusting the final volume to 1 liter; this solution is listed as Solution A in table 4; Solution B was obtained by diluting Solution A to one-fifth strength. (2) A solution of mixed organic acids prepared by dissolving citric, malic, tartaric and oxalic acids (0.7 g. of each acid) in somewhat less than 1 liter of H<sub>2</sub>O, adding NaOH until approximately pH 6 was obtained, and adjusting the final volume to 1 liter. This is listed as Solution C in table 4. Solution D was prepared from Solution C by diluting to one-fourth strength.

TABLE 4. *Acidification of Organic Acid Solutions by the Addition of Salts*

Salt Added	M Conc.	pH Observed After Adding Salt to:			
		Malic Acid—Sodium Malate Solutions		Mixture of Citric, Malic, Tartaric and Oxalic Acids	
		Solution A*	Solution B*	Solution C*	Solution D*
None KCl	H <sub>2</sub> O, Check	3.41	3.50	6.09	6.41
	0.1	3.39	—	5.92	6.29
	0.025	3.41	—	—	—
	0.01	3.41	—	6.14	6.25
	0.0025	3.42	—	—	—
CaCl <sub>2</sub>	0.001	3.42	—	—	—
	0.1	3.03	3.06	4.78	5.46
	0.025	3.28	3.30	—	—
	0.01	3.36	3.39	5.46	5.85
	0.0025	3.40	3.47	—	—
CuSO <sub>4</sub>	0.001	3.40	3.50	5.98	6.17
	0.1	2.55	2.62	3.24	3.45
	0.025	3.04	2.85	—	—
	0.01	3.24	3.06	4.24	3.78
	0.0025	3.38	3.34	—	—
	0.001	3.40	3.45	6.00	5.58

\* The composition of these solutions is described in the text.

To 10 cc. samples of these liquids 1 cc. of 1.0 *M*, 0.1 *M* and 0.01 *M* KCl, CaCl<sub>2</sub>, and CuSO<sub>4</sub> solutions were added, thus making the solutions 0.1 *M*, 0.01 *M* and 0.001 *M* with respect to the salts. The pH of the liquids was then determined and the results are shown in table 4. The data in this table show that acidifications were obtained with CaCl<sub>2</sub> and CuSO<sub>4</sub> even with the diluted solutions.

Solution A when diluted 1 : 1 gives a liquid with approximately the same pH and titration curve as apple juice, and Solution C when diluted 1 : 1 gives a liquid with approximately the same pH and titration curve as the water-extract of potato tissue. And, although we do not claim that these organic acid mixtures represent the actual composition of the juice or extract, the data are competent to show that acidifications can be obtained with them even when the concentrations are lower than those required to give a titration curve comparable to that obtained with the plant juices or extracts.



What are the causes of the acidifications that are produced when salts such as calcium chlorid and copper sulphate are added to organic acids? Precipitation of insoluble salts of organic acids, *e.g.* calcium oxalate, is an important factor. This is shown in text figure 2. In such cases the concentration of the organic acid radical is reduced; consequently the repressing effect upon the ionization of the organic acid is lessened, and an increase in hydrogen-ion results.

However, we obtained acidifying effects in cases where the addition of the salt did not produce precipitation, *e.g.*  $\text{CaCl}_2$  and  $\text{CuSO}_4$  to malic, succinic, and acetic acids (text fig. 2). The true causes of the acidifications in all these cases are not clear; we may be dealing with the effect of the salt upon the activity of the hydrogen-ion discussed by Michaelis (5, vol. 1, p. 130), or with the salt effect upon buffers discussed by Haynes (3, p. 448); we may also have complex-ion or salt formation between the cation and the organic acid especially in the case of copper ions which are known to form complexes with a number of organic acids, *e.g.* copper and tartaric acid in Fehling's solution, and to a certain extent in the case of calcium which according to Treadwell-Hall (11, vol. 1, p. 388) forms a complex-ion with tartaric acid.

But whatever may be the factors causing these increases in hydrogen-ion concentration upon the addition of salts to organic acids, the essential point in connection with the present discussion is that they occur, and that the changes in pH are in the same direction and of the same order of magnitude as those obtained by bringing the salts in contact with the tissue itself.

### Effect of Adding Calcium Chlorid to Phosphate Solutions

The acidity resulting from the addition of  $\text{CaCl}_2$  to solutions containing phosphates is shown in text figure 3. To 10 cc. samples of phosphoric acid solutions were added varying amounts of alkali to give a series of solutions with different pH values; in each case the volume was adjusted to 20 cc. by the addition of water; this gave phosphate solutions of the concentrations shown in text figure 3; each of these samples was then divided into two equal parts, to one of which 1 cc. of 1.0 *M*  $\text{CaCl}_2$  solution was added and to the other 1 cc. of water was added. The resulting pH values are shown in text figure 3. It is seen that acidifications were produced by the addition of  $\text{CaCl}_2$  when the hydrogen-ion concentration was more alkaline than about pH 4 to 5. At hydrogen-ion concentrations more acid than pH 4 to 5 no precipitate was formed and no change in pH resulted from the addition of  $\text{CaCl}_2$ .

From the results shown in text figure 3 we should expect that phosphates, if present in plant extracts in sufficient concentration, would cause acidification upon the addition of  $\text{CaCl}_2$ , because of the precipitations that occur when the acidity of the solution attains certain values.

### Effect of Addition of Calcium Chlorid to Pectin Solution

In this experiment a 1-percent pectin solution was used. The procedure was as follows: to 10 cc. of pectin solution varying amounts of acid and alkali were added to produce solutions of different hydrogen-ion concentrations; the volume was then adjusted to 20 cc. by the addition of water; the solution so obtained was divided into two equal parts, to one of which 1 cc. of 1.0 *M* CaCl<sub>2</sub> solution was added and to the other 1 cc. of water. The pH values were measured and the results are shown in table 5.

TABLE 5. *Effect of Addition of Calcium Chlorid to Solutions of Pectin and Solutions of Soluble Egg Albumen\**

Pectin Solution:			Egg Albumen Solution:		
Cc. of 0.02 N Acid or Alkali Added	pH After Adding:		Cc. of 0.1 N Acid or Alkali Added	pH After Adding:	
	1 cc. H <sub>2</sub> O	1 cc. CaCl <sub>2</sub>		1 cc. H <sub>2</sub> O	1 cc. CaCl <sub>2</sub>
8 cc. HCl	2.45	2.35	3 cc. HCl	2.75	2.92
4 " "	2.94	2.67	2 " "	3.36	3.55
1 " "	3.63	3.09	1 " "	4.31	4.30
0 — —	4.03	3.33	0.5 " "	4.99	4.83
1 cc. NaOH	4.38	3.61	0.25 " "	5.68	5.34
2 " "	5.02	4.38	0 — —	6.47	6.03
3 " "	6.42	6.56	0.5 cc. NaOH	8.7	8.3
4 " "	7.84	7.41			
6 " "	8.39	7.88			

\* Pectin solution = 1 percent; egg albumen solution = 2 percent; 10 cc. of solution + given amount of acid or alkali + H<sub>2</sub>O to make 20 cc. Solution divided into two parts and 1 cc. CaCl<sub>2</sub> (1.0 *M*) or 1 cc. H<sub>2</sub>O added.

It is seen that the addition of calcium chlorid increased the acidity in all cases but one.

### Effect of the Addition of Calcium Chlorid to Protein Solutions \*

The view that the proteins in seeds or other plant tissues produce these effects upon the hydrogen-ion concentrations of salt solutions may have resulted from attempting to apply under these conditions the principle of Loeb (4) that a protein can combine only with cations when the pH of the solution is more alkaline than that at the isoelectric point of the protein and only with anions when the pH of the solution is more acid.

When salts are added to protein solutions, however, it must not be assumed that a large change in hydrogen-ion concentration must result. Loeb (4, p. 101) showed that the addition of NaCl to gelatin at pH 3.0 did not change the pH. We carried out an experiment with 1.6 percent gelatin which had been purified by the Loeb method (4, p. 35) and which had been brought to various pH values by the addition of NaOH and HCl. Samples consisting of 20 cc. of solutions so obtained were divided into two parts,

to one of which 1 cc. of 1.0  $M$   $\text{CaCl}_2$  was added and to the other 1 cc. of  $\text{H}_2\text{O}$ . The pH values were then obtained and are shown in the gelatin curve, text figure 4. The results show that only small changes in the pH values resulted from the addition of  $\text{CaCl}_2$ ; on the acid side of the isoelectric point of gelatin (pH 4.6) we can not be sure from the data obtained that the addition of  $\text{CaCl}_2$  produced any change; on the alkaline side of the isoelectric point the acidity was slightly increased.

The results with a solution of egg albumen are shown in table 5. The solution used was a 2-percent solution of commercial soluble egg albumen. The data show that at a pH of about 4.3 no effect was produced by the addition of  $\text{CaCl}_2$ , that at pH values more acid than 4.3  $\text{CaCl}_2$  made the solution more alkaline and at pH values more alkaline than 4.3 the solution became more acid. The behavior of the egg albumen solution was not similar, therefore, to the behavior of plant tissue or tissue extracts, since in no case have we observed a development of alkalinity upon the addition of calcium chlorid to tissues or tissue extracts.

Text figure 4 shows the results that were obtained with the non-dialyzable fraction of potato juice which according to Cohn, Gross and Johnson (2) contains tuberin, the principal protein of potato; no doubt other colloidal substances were also present. After dialyzing potato juice by means of collodion bags, the portion not passing through the membrane was stirred by means of a mechanical stirrer and 10 cc. samples were removed; varying amounts of acid and alkali were added as shown in text figure 4; the volume was then adjusted to 20 cc. by the addition of  $\text{H}_2\text{O}$ ; the solution was divided into two equal parts to one of which 1 cc. of 1.0  $M$   $\text{CaCl}_2$  solution was added and to the other 1 cc. of  $\text{H}_2\text{O}$ . The pH values that were obtained are shown in text figure 4. Although slight acidifications were produced by the addition of  $\text{CaCl}_2$  at pH values more alkaline than pH 5.0, and reductions in hydrogen-ion concentrations within the range pH 3. to 4, it is seen that the curves representing the data obtained before and after the addition of  $\text{CaCl}_2$  are practically identical.

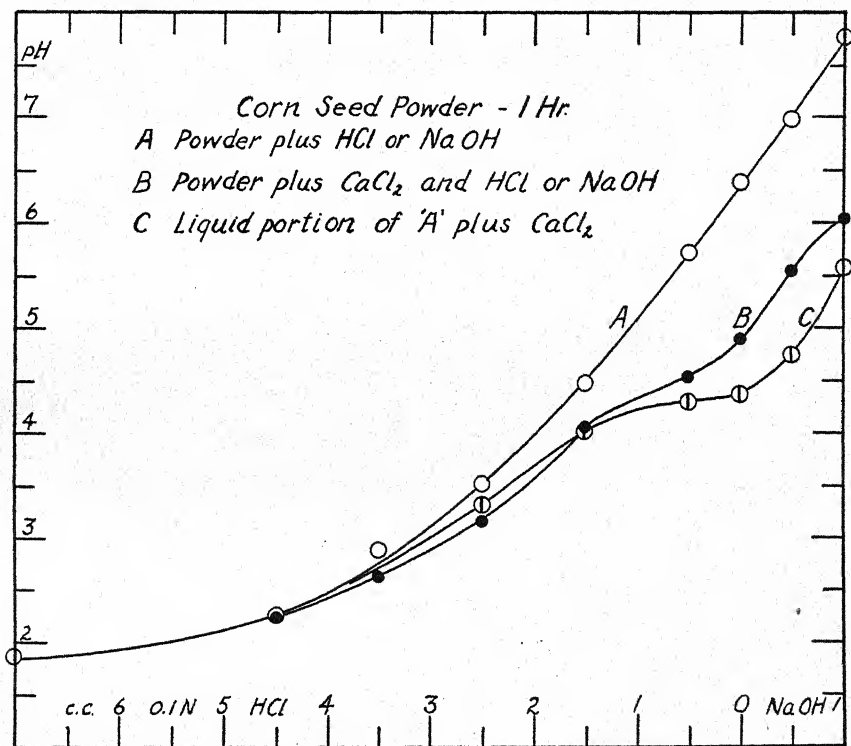
In contrast to the behavior of these protein or protein-containing solutions is that of the dialyzable portion of potato juice shown in text figure 4, and the organic acid and phosphate solutions shown in text figures 2, 3, and 4. It is important to note that the results obtained with these liquids were comparable to those obtained with the tissues but that the protein or protein-containing fractions showed a behavior not analogous to that of the tissue.

#### Effects of Addition of Calcium Chlorid to Corn Seed Powder after Adjusting the External Solution to Various pH Values

Corn seeds (*Zea mays*) were ground to a fine powder; 2-gram samples were weighed out into test tubes and acid or alkali added to bring the external solution to various pH values. There were two test tubes con-

taining equal amounts of powder and equal amounts of acid or alkali for each pH value at which the test was carried out. To one lot 1 cc. of 1.0  $M$   $\text{CaCl}_2$  was added, to the other 1 cc. of  $\text{H}_2\text{O}$ . The volume was then adjusted to 20 cc. and the tubes were rotated end-for-end on a turning-bar for 1.5 hours; the samples of the supernatant liquid were then removed and the pH value determined.

The object of the experiment was to bring the proteins in the seed powder to different pH values in order to note whether a pH value could be noticed on one side of which the calcium chlorid would react in one way, and on the other side in a different way.



TEXT FIG. 5. Curves showing the lack of evidence of an isoelectric point on one side of which the tissue reacted with cations and on the other side with anions. Addition of  $\text{CaCl}_2$  caused acidifications at all pH values from 2.5 to 7.5. Compare the flat portion of curves B and C between pH 4 and 5 with the phosphate curves in figure 3.

The results are shown in curves A and B, text figure 5. Acidifications by adding  $\text{CaCl}_2$  to the powder were produced at all pH values from 2.5 to 7.5; no pH value was noted on one side of which the action of calcium chlorid differed materially from that on the other side. There is no evidence of an isoelectric point on one side of which the tissue reacted with cations and on the other side of which it reacted with anions.

Curve *C*, text figure 5, was obtained by centrifuging the lots to which calcium chlorid had not been added, decanting off the extract and then adding an equivalent amount of  $\text{CaCl}_2$  to the extract. Acidifications were produced at pH values more alkaline than about 2.5; marked increases in  $\text{H}^+$  were shown at pH values more alkaline than 4.2.

Curves *B* and *C* in text figure 5 show a bulge or hump at about pH 4.25. We have repeated this experiment and are certain that this hump is not due to experimental error. Furthermore, in testing this point we made use of wheat seed powder to carry out a similar experiment. There is a similar bulge in the curve for wheat, the hump occurring, however, at about 4.5. While we do not know all the factors which produce this shape of curve under the conditions of this experiment, the results obtained with phosphate solutions shown in text figure 3, and with organic acid solutions shown in text figure 2, indicate that these substances take part in the reactions which cause the curve to take this particular form.

### DISCUSSION

Although we believe that the changes in the pH of the external solution upon the addition of tissues to salt solutions under the conditions of our experiments are not caused mainly by the unequal absorption of ions by the tissue, we do not wish to maintain that the tissue does not absorb ions, nor that an unequal absorption does not take place. It has been shown by others (Stiles, 10) that cations may be removed from the external solution faster than anions; however the pH need not be changed by this process since an equivalent amount of other cations may leach out of the tissue. Stiles (10, p. 622) showed in the case of carrot tissue in  $\text{NaCl}$  that Na was removed faster than Cl, but the hydrogen-ion concentration did not change during this process because of the replacement of Na by other ions which passed into the external solution from the tissue.

We do not claim that the mixtures of organic acids, or the phosphate or pectin solutions that we have used, represent the actual chemical composition of the juices or water-extracts of the tissues. They have been used merely as illustrative material to indicate that they are factors in these pH changes. The extent to which each may occur in the juices or water-extracts and the part that each may take quantitatively in producing the observed changes would require a separate investigation involving a chemical analysis of the various tissues and tissue extracts.

### SUMMARY

This is a continuation of the experiments relating to the question whether plant tissue, in its effect upon the pH of the surrounding solution, acts as though it had an isoelectric point, or shows a behavior analogous to that of a protein. A previous paper dealt with the effects of tissues upon the pH of buffer solutions (phosphate, phthalate, and borate); this is a report of a



series of experiments in which unbuffered salt solutions (KCl, CaCl<sub>2</sub>, and others) were used.

Plant tissues, such as thin disks of potato tubers, carrot roots, or apple fruits, whole seeds of corn, rye and wheat, and corn seed-powder were placed in salt solutions the concentration of which was varied from 0.1 *M* to 0.001 *M*; samples of the external liquid were removed at intervals and the pH value was determined.

Previous reports by others that plant tissues, when placed in different salt solutions of different concentrations, are able to bring the external pH to definite values characteristic for each kind of tissue, were not confirmed. In our experiments the external pH value varied with the salts used, with the concentration of the salt, and especially with the nature of the cation. Only when the concentration of a salt was sufficiently reduced was a characteristic pH value for a given tissue approached, and this pH value was merely the pH of the water-extract of the tissue obtained by bringing the tissue in contact with water instead of salt solution.

The changes in hydrogen-ion concentration of the external solution (when any changes were obtained) were always in the direction of increased acidity. This effect was less marked in the case of salts with monovalent cations such as K and Na; but acidification was observed in every case with salts having divalent cations such as Ca and Cu. The increases in acidity when plant tissues were placed in CaCl<sub>2</sub> solutions were strikingly great, the hydrogen-ion concentration increasing ten, twenty or thirty fold under suitable experimental conditions.

It is shown that this increase in H<sup>+</sup> is not to be interpreted as being the result of unequal rate of absorption of cations and anions by the tissue. We found that similar acidifications were produced by adding the salts to the water-extract obtained by bringing the tissue in contact with water instead of salt solution. In these cases the salt came in contact not with the tissue itself but only with the soluble substances that leached out of the tissue. In addition, when the water-extracts were boiled to eliminate the proteins coagulable by heat, and when the water-extracts were subjected to dialysis, it was found that the non-coagulable, non-colloidal and non-protein fractions underwent acidification upon the addition of salts, giving results analogous to those obtained by bringing the salts in contact with the tissues themselves.

Furthermore, we found that increases in hydrogen-ion concentration were obtained when salts were added to various organic acids such as malic, oxalic, succinic, aspartic, etc. When the organic acids were first adjusted to various pH values before the addition of CaCl<sub>2</sub>, acidification effects were observed at all pH values from 2 to 7.5. Precipitation of insoluble salts of organic acids with consequent increase in ionization of the remaining acid were shown to account in part for this development of acidity on adding salt solutions to organic acids; but acidifications were also observed in

cases in which no precipitation occurred. The causes operating in such cases are briefly discussed in the text.

Acidifications were also observed when  $\text{CaCl}_2$  was added to solutions of phosphates and pectin.

Experiments in which  $\text{CaCl}_2$  was added to protein solutions at various pH values and to that fraction of potato juice containing the protein tuberin, showed that the behavior of proteins on the addition of salts was not analogous to that obtained on adding salts to the tissues.

Cases were observed in which the total effect of the tissue could not be accounted for by the substances leaching out of the tissue and reacting with the salt solution. It is shown, however, that this effect (which may be that of adsorption by constituents of the tissue) need not be ascribed to proteins since similar results were obtained with tissue containing no protein.

Although our results do not show that the tissue itself or the proteins take no part whatever in these changes in pH, they indicate that the soluble, non-protein, non-colloidal substances which diffuse out of the tissue into the salt solution and which then react with it are important factors in the acidifications that are produced in the external solution.

The observed changes in hydrogen-ion concentration, therefore, cannot be interpreted as indicating an isoelectric point for the tissue as a whole, nor furnish proof that reaction has occurred between the ions of the salt solution and proteins with characteristic isoelectric points.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.,  
YONKERS, NEW YORK

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# TOMATO MOSAIC. FILTRATION AND INOCULATION EXPERIMENTS

H. R. KRAYBILL AND S. H. ECKERSON

(Received for publication August 15, 1927)<sup>1</sup>

In order to learn more about the infective principle of tomato mosaic, the juice of mosaic plants was separated by filtration methods and healthy tomato plants were inoculated with the different fractions.

## METHODS

The method of inoculation described under experiment 2 proved satisfactory in every way and was used in most of the work. In the preparation of materials for inoculation, the preliminary treatment was the same in all cases: the plants were ground in a Nixtamal mill and the juice was pressed out by means of a tincture press. Then the juice was either filtered without further treatment, or the colloidal substances were partially removed before filtration. After filtration all preparations were stored in stoppered Erlenmeyer flasks with a layer of toluol over the surface of the liquid. The filters used were fritted glass filters<sup>2</sup> numbers 3/<7, 4/<7, and 6/<7, and collodion filters of different densities.

## RESULTS

With vigorous young plants and good methods of inoculation the residues from glass filters usually gave 100 percent mottling after a single inoculation, while the filtrates produced no mottling symptoms (tables 2, 3). Apparently the mottling principle had been filtered out, yet there was some rather indefinite effect of the inoculation. It seemed possible that more definite symptoms might develop if the dose were increased. Accordingly young plants were inoculated with the filtrate three times at two-day intervals. Approximately 50 to 75 percent of the plants developed "fern-leaf"<sup>3</sup> symptoms but not the slightest indication of mottling (tables 5 and 6 and Pls. LXIII and LXIV).

<sup>1</sup> Published, at the expense of the Boyce Thompson Institute for Plant Research, out of the order determined by the date of receipt of the manuscript.

<sup>2</sup> The fritted glass filters employed in these studies are described in a circular published by the Empire Laboratory Supply Company. They are made by fusing granulated Jena glass. The size of the pores is controlled by the size of the particles of glass used. The filters used in these experiments were supposed to have pores from 4 to 5 microns in diameter.

<sup>3</sup> The fern-leaf plants were extremely dwarfed, bearing small, comparatively smooth, and glistening leaves. Many of the leaflets were deeply cut owing to the slight development of leaf-blade tissue between the veins. The general appearance of leaves and plants of this type are shown in Plate LXIII, figure 1, and Plate LXIV, figure 3.



However, when the colloidal materials were removed from the plant juice before filtering (by centrifuging, digestion with enzymes, or other means) the mottling principle passed through the glass filters (tables 4, 5, 6) but was filtered out by collodion membranes (table 6). The filtrates from the glass filters, which contained the mottling principle, produced only fern leaf after filtration through collodion membranes.

### Filtration Without Previous Removal of Colloidal Substances

#### *Series A*

During the last week in September, 1925, several dozen tomato plants showing marked symptoms of the mosaic disease were secured from a tomato field adjacent to the Institute gardens. The juice was allowed to stand at room temperature for several weeks. On October 15 the juice, which had fermented, was filtered through a fritted glass filter number 3/<7 into a suction flask attached to a water pump. The filtrate was designated inoculum 1A. The residue upon the filter was suspended in water and labeled 5A. A portion of 1A was filtered through a collodion membrane through which 208 cc. of distilled water passed in one hour when under the reduced pressure of a water suction pump. This filtrate was designated 2A. A portion of 2A was then filtered through a collodion membrane through which 178 cc. of distilled water passed in one hour when under the reduced pressure of a water suction pump. This filtrate was labeled 3A. A portion of 3A was accidentally diluted with about five parts of tap water and this formed the inoculum 4A.

*Experiment 1:* Bonny Best tomato plants about six inches high planted in soil in four-inch pots were used for the experiment. Half of the plants in each lot were inoculated on the upper side of the leaf and half on the lower side of the leaf. Inoculations were made on about five leaflets of each plant. The hairs were shaved off by means of a razor and then the inoculum was swabbed on by means of a small plug of cotton attached to a glass rod. These plants were inoculated on October 27. Table I presents the results of the experiment.

TABLE I

Number of Plants	Inoculum Used	Apparently Healthy Plants	Mosaic Plants
8	1A, filtrate, glass filter	8	0
8	2A, filtrate, collodion	8	0
8	3A, filtrate, collodion	8	0
8	4A, filtrate, collodion	8	0
8	5A, residue, glass filter	6	2
12	Controls	12	0

*Experiment 2:* The plants in this experiment were inoculated by means of scoring the leaf with a steel needle and then placing a drop of the preparation on the leaf with a medicine dropper. The material was then introduced

into the tissue by further scoring with the needle. The needle was sterilized before inoculating each plant by heating in an alcohol flame. The leaf was held by means of a small piece of paper toweling which was discarded for a new piece after each plant was inoculated. Table 2 presents the results of this experiment. These plants were inoculated on November 23 and again two days later.

TABLE 2

Number of Plants	Condition of Plants at Beginning of Experiment	Inoculum Used	Number of Apparently Healthy Plants	Number of Mosaic Plants
6	Pot-bound	1A, filtrate, glass filter	6	0
6	"	2A, filtrate, collodion	6	0
6	"	3A, filtrate, collodion	6	0
6	"	4A, filtrate, collodion	6	0
6	"	5A, residue, glass filter	6	0
10	"	Controls	10	0
5	Young vigorous plants	1A, filtrate, glass filter	5	0
5	"	5A, residue, glass filter	0	5
10	"	Controls	10	0

*Series B*

On December 8, 1925, eight tomato plants which showed marked mosaic symptoms were secured from a nutrition experiment in the greenhouse. The juice was filtered through a fritted glass filter number 6/<7 by means of reduced pressure from a water pump. The filtrate (500 cc.) was labeled 1B. The residue upon the filter was suspended in 150 cc. of distilled water and designated 5B. The preparations were then stored in a manner similar to those of series A.

On January 28, 1926 one half (about 70 cc.) of preparation 5B was transferred to a fritted glass filter number 6/<7 and washed with distilled water. The first 400 cc. of filtrate formed preparation 6B; the next 600 cc. 8B, and the last 50 cc. 9B. The residue was then suspended in about 70 cc. of water and labeled 7B (note table 4).

*Experiment 3:* Vigorous tomato plants of the Stone variety about six inches high were used for this experiment. Inoculations were made December 23, in the same manner as in experiment 2. The results of the experiment are given in table 3.

TABLE 3

Number of Plants	Inoculum Used	Number of Apparently Healthy Plants	Number of Mosaic Plants
6	5A, residue, glass filter	0	6
6	1A, filtrate, glass filter	5	1
6	5B, residue, glass filter	0	6
6	1B, filtrate, glass filter	6	0
12	Controls	11	1

## Filtration After Partial Removal of Colloidal Substances

*Series E*

Six mosaic plants from inoculation experiment 2 and seven plants (which showed marked symptoms of mosaic) from a nutrition experiment were taken January 26. Toluol was placed over the juice as soon as it was expressed from the plants. A portion of the juice was filtered through fritted glass filter number 4/<7. The filtrate was labeled 1E. The residue was suspended in water and labeled 2E. Another portion of the juice was centrifuged and the liquid was then filtered through fritted glass filter number 3/<7. This filtrate was designated 3E. The residue on the filter was diluted with water and labeled 4E. The residue in the bottom of the centrifuge tubes was diluted with about 100 cc. of water and labeled 5E. A portion of the liquid, which had been centrifuged, was allowed to stand overnight and then was centrifuged again. The light green cakes on top of the tubes were suspended in 100 cc. of water and labeled 6E. The liquid portion of the material centrifuged was labeled 7E and the residue in the tubes was suspended in 100 cc. of water and labeled 8E. A portion of 7E was filtered through fritted glass filter number 6/<7 and the filtrate labeled 9E. The residue upon the filter was designated 10E.

*Experiment 4:* Vigorous plants of the Stone variety about four inches high, which had been transplanted to six-inch pots, were used for this experiment. Inoculations were made by pricking the stem close to the tip with a sterilized needle and then dropping the inoculum on to the injured part of the stem by means of a medicine dropper and introducing the material into the stem by subsequent scratching of the tissue with the needle. The plants received only one inoculation. Table 4 shows the results of the experiment.

TABLE 4

Number of Plants	Inoculum Used	Number of Apparently Healthy Plants	Number of Mosaic Plants
12	5B, residue, glass filter	8	4
12	7B, 5B washed over filter	11	1
12	6B, washings from 7B	12	0
12	8B, washings from 7B	12	0
7	Controls	7	0
11	9B, washings from 7B	11	0
17	Controls	17	0
14	1E, filtrate, glass filter	14	0
14	2E, residue, glass filter	8	6
14	3E, centrifuged filtrate, glass filter	12	2
14	4E, centrifuged residue, glass filter	11	3
14	5E, residue at bottom of centrifuge	9	5
13	6E, cakes on top of centrifuge	10	3
15	7E, liquid from centrifuge	2	13
14	8E, residue at bottom of centrifuge	8	6
14	9E, filtrate of 7E through glass filter	5	9
14	10E, residue, glass filter	4	10
37	Controls	37	0
14	1A, filtrate, glass filter	13	1
14	2A, 1A filtered through collodion	14	0

*Series I*

On March 30 the juice was expressed from the stems of mosaic plants from experiment 4. Trypsin and takadiastase were added to the 125 cc. of juice which was allowed to stand at room temperature with toluol over the surface until April 1. On April 1 the juice was centrifuged and the clear extract was decanted off and allowed to stand for several days at room temperature with a layer of toluol on the surface. It was then filtered through fritted glass filter number 4/<7 into a test tube inside of a suction flask. The entire equipment had been sterilized by heating in an oven at 140° C. for 5 days. The filtrate was labeled 1I and was kept sterile in a cotton-plugged test tube. Portions of the filtrate were plated out on six different bacteria culture media and after ten days the results indicated that the filtrate was sterile.

*Experiment 5:* Bonny Best tomato plants about four inches high planted in four-inch pots were used for this experiment. These, and all later inoculations, were made as described in experiment 2. Table 5 presents the results of the experiment, as do Plate LXIII, figure 1, and Plate LXIV, figure 3.

*Series L*

The juice was expressed from a large number of mosaic plants from experiment 4. The 1,000 cc. of juice obtained was diluted with 1,000 cc. of distilled water and placed in an ice box until April 28. The liquid portion was decanted off the top and the following separations were made: 1L is filtrate through fritted glass filters numbers 4/<7 and 6/<7, which was then passed successively through a series of five S. and S. Bechold acetic acid collodion filters of increasing density, giving filtrates 2L, 3L, 4L, 5L, and 6L; 7L is residue from fritted glass filter diluted with 150 cc. of water; 8L is half of 7L washed with water through fritted glass filter 6/<7; 9L is first filtrate of about 75 cc. of water from 8L.

About 600 cc. more of liquid was decanted from the original juice and the following separations were made: 10L is the filtrate from fritted glass filter 6/<7, and 11L is the residue on the filter suspended in water. The filtrate 10L, from the glass filter, was filtered separately through three grades of collodion filters giving filtrates 12L, 13L, and 14L.

*Series M*

The juice was expressed from 14 plants from experiment 5, which had been inoculated with preparation 1A or 3A and which showed fern leaf symptoms. Separations were made as follows: the juice was allowed to settle from morning until evening and then decanted off liquid; the liquid portion is 1M; 2M is residue from filtering 1M through fritted glass filter 6/<7; 3M is filtrate from filtering 1M through fritted glass filter 6/<7; 4M is residue which settled to bottom of beaker when 1M was decanted off.

The residue was washed several times on filter 6/<7 and then diluted with 50 cc. of water.

TABLE 5

Number of Plants	Number of Times Inoculated	Inoculum Used	Apparently Healthy Plants	Mottled Plants	Fern-leaf Plants
18	2	<i>x</i> , sterile filtered tobacco mosaic	14	4	0
18	3	1A, filtrate, glass filter	4	0	14
18	3	3A, 1A through collodion	6	0	12
18	3	5A, residue, glass filter	4	14	0
18	3	1B, filtrate, glass filter	15	0	3
9	2	5B, residue, glass filter	0	9	0
9	2	7B, 5B washed with water	6	3	0
9	2	1I, centrifuged juice, filtrate from glass filter	0	9	0
125	0	Controls	125	0	0

*Experiment 6:* One plant from experiment 5, which showed marked mottling symptoms of mosaic, was ground in the mill. Another plant which showed fern-leaf symptoms only was extracted in the usual manner. Nine plants were inoculated with the juice from the plant showing the mottling symptoms. All of these plants developed definite symptoms of mosaic. Nine plants were inoculated with the juice from the plant showing fern-leaf symptoms. Four of these developed definite mosaic symptoms.

*Experiment 7:* Plants 2 to 4 inches high were inoculated May 12 and 14. Table 6 shows the results of the experiment, as do figures 2 and 4.

TABLE 6

Number of Plants	Number of Times Inoculated	Inoculum Used	Apparently Healthy Plants	Mosaic Plants	Fern-leaf Plants
8	2	1A, filtrate, glass filter	3	0	5
8	2	2A, filtrate from glass filter, through collodion	4	0	4
8	2	1B, filtrate, glass filter	4	0	4
8	2	5A, residue, glass filter	2	6	0
8	2	1E, filtrate, glass filter	8	0	0
8	2	2E, residue, glass filter	0	8	0
8	2	1M, liquid from fern-leaf plants	0	8	0
8	1	2M, residue, glass filter	0	8	0
8	1	3M, filtrate, glass filter	4	4	0
8	1	4M, residue settled out	6	2	0
8	2	1L, filtrate, glass filter	3	2	3
8	2	2L, filtrate 1L through 1½% collodion	0	0	8
8	2	3L, filtrate 2L through 3% collodion	7	0	1
8	2	4L, filtrate 3L through 4½% collodion	6	0	2
8	2	5L, filtrate 4L through 6% collodion	4	0	4
8	2	6L, filtrate 5L through 7½% collodion	4	0	4
8	1	10L, filtrate, glass filter	1	7	0
8	1	11L, residue, glass filter	0	8	0
8	1	12L, filtrate 10L through 1½% collodion	7	0	1
8	1	13L, filtrate 10L through 3% collodion	5	0	3
8	1	14L, filtrate 10L through 4½% collodion	8	0	0

## Series N

Plants were selected from experiment 7 and ground separately. The Nixtamal mill and beakers were sterilized by boiling in water before expressing the juice from each plant. The following preparations were made: 1N from a plant with moderate fern-leaf symptoms of inoculation 2F; 2N from a plant with marked fern-leaf symptoms of inoculation 2A; 3N from a plant with marked fern-leaf symptoms of inoculation 1A; 4N from a plant with marked fern-leaf symptoms of inoculation 2; 5N from a plant with marked fern-leaf symptoms of inoculation 1; 6N from a plant with slight fern-leaf symptoms of inoculation 5L; 7N from a plant with slight fern-leaf symptoms of inoculation 5L; 8N from a plant with slight fern-leaf symptoms of inoculation 13L; 9N, 10N, 11N, 12N, 13N, and 14N from control plants with no symptoms of disease.

*Experiment 8:* Plants about four inches high were inoculated. The results are shown in table 7.

TABLE 7

Number of Plants	Inoculum Used	Healthy Plants	Mosaic Plants
6	1N, plant having moderate fern-leaf symptoms	6	0
6	2N, plant having marked fern-leaf symptoms	6	0
6	3N, plant having marked fern-leaf symptoms	6	0
6	4N, plant having marked fern-leaf symptoms	6	0
6	5N, plant having marked fern-leaf symptoms	6	0
6	6N, plant having slight fern-leaf symptoms	6	0
6	7N, plant having slight fern-leaf symptoms	5	1
6	8N, plant having slight fern-leaf symptoms	6	0
6	9N, plant apparently healthy	6	0
6	10N, plant apparently healthy	6	0
6	11N, plant apparently healthy	6	0
6	12N, plant apparently healthy	2	4
6	13N, plant apparently healthy	1	5
6	14N, plant apparently healthy	6	0

## DISCUSSION

When the juice from mosaic tomato plants was passed through the glass filters (preparations 1A, 1B, 1E) without previously centrifuging or allowing the juice to stand in an ice box to remove colloidal constituents, the infectious principle which produces mottling was retained with the residue on the filter. When the mosaic juice was allowed to stand in the ice box until the chlorophyll and a large part of the proteins settled out (preparations 1L and 10L); when the juice was centrifuged to remove some of the colloidal material (preparations 3E, 9E and 11); or when the juice contained very little colloidal matter (preparation 3M), the infectious principle, which produces mottling, passed through the glass filter into the filtrate. Tests with cultures of *Bacterium tumefaciens* showed that these bacteria passed through the glass filters readily. If, however, the culture solution was filtered through a filter which had upon it a layer of the residue

from the filtration of some of the tomato juice, the bacteria were retained on the filter and none passed through with the filtrate. The removal of the infectious principle of the mosaic juice by the glass filters, which have pores 4-5 microns in diameter, is due to the layer of material which collects on the filter.

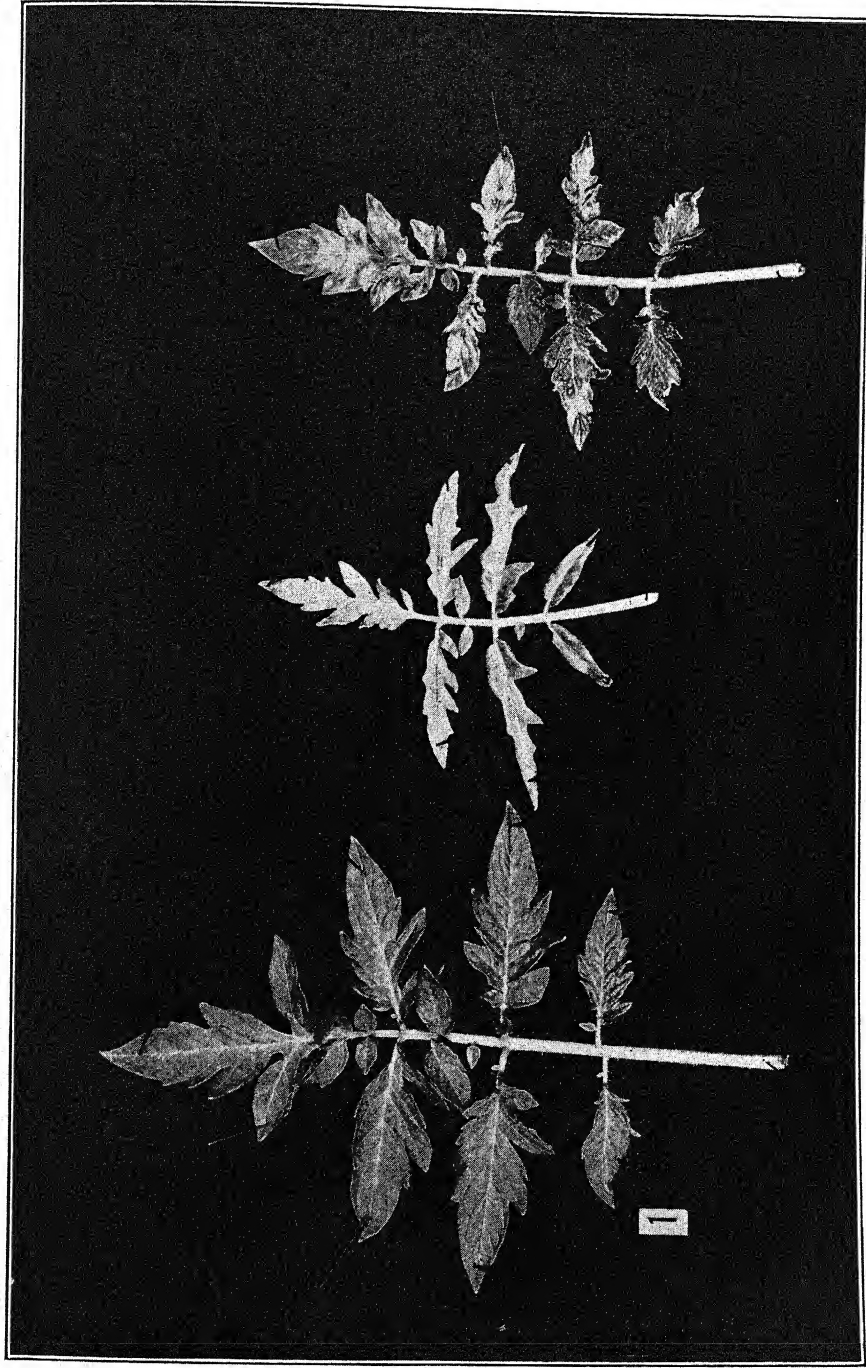
When filtrates from glass filters which contained the infectious principle which produces mottling symptoms (preparations *1L* and *10L*) were passed through collodion membranes, the infectious principle was removed and the filtrates (preparations *2L*, *3L*, *4L*, *5L*, *6L*, *12L*, *13L* and *14L*) failed to produce mottling symptoms.

The filtrates from the glass filters (preparations *1A* and *1B*) produced fern-leaf symptoms without any mottling. When passed through collodion membranes, these filtrates were still effective in producing fern-leaf symptoms. When preparations *1L* and *10L*, filtrates from glass filters which produced mottling symptoms, were passed through collodion membranes they no longer produced mottling symptoms but produced fern-leaf symptoms.

Attempts to transfer the fern-leaf type of symptom to other plants by inoculation with juice from plants showing only the fern-leaf symptoms were unsuccessful. In experiments 6 and 7 mottling symptoms were obtained by inoculating with the juice from plants showing only fern-leaf symptoms. The results of experiment 8 and later experiments indicate that the fern-leaf plants of experiments 6 and 7 carried the typical tomato mosaic also. It is quite probable that one or more plants were infected accidentally late in the experiment and had not yet developed the mottling symptoms. When single plants were used in attempting to transfer the fern-leaf symptoms (experiment 8) no more mosaic was found in plants inoculated with juice from fern-leaf plants than in those inoculated with juice from the apparently healthy plants. Some of the fern-leaf plants, when kept for several months, seemed to recover and outgrow the effects of the inoculation.

By means of glass filters juice from the mosaic tomato plants was separated into two fractions, a residue which contained the infectious principle (probably an organism) which produces mottling symptoms, and the filtrate which contains substances (probably of the nature of toxins) which produce fern-leaf symptoms. This does not seem to be infectious. Whether the substances in the filtrate producing the fern-leaf symptoms are formed by the decomposition of some compounds of the plant and have no relation to the mosaic, or whether they are formed by the agent causing the mosaic disease, must be determined by further studies. At least this much is certain, the symptoms produced are very similar to the fern-leaf and filiform symptoms frequently associated with the mosaic disease and cannot readily be differentiated from those symptoms of mosaic plants.

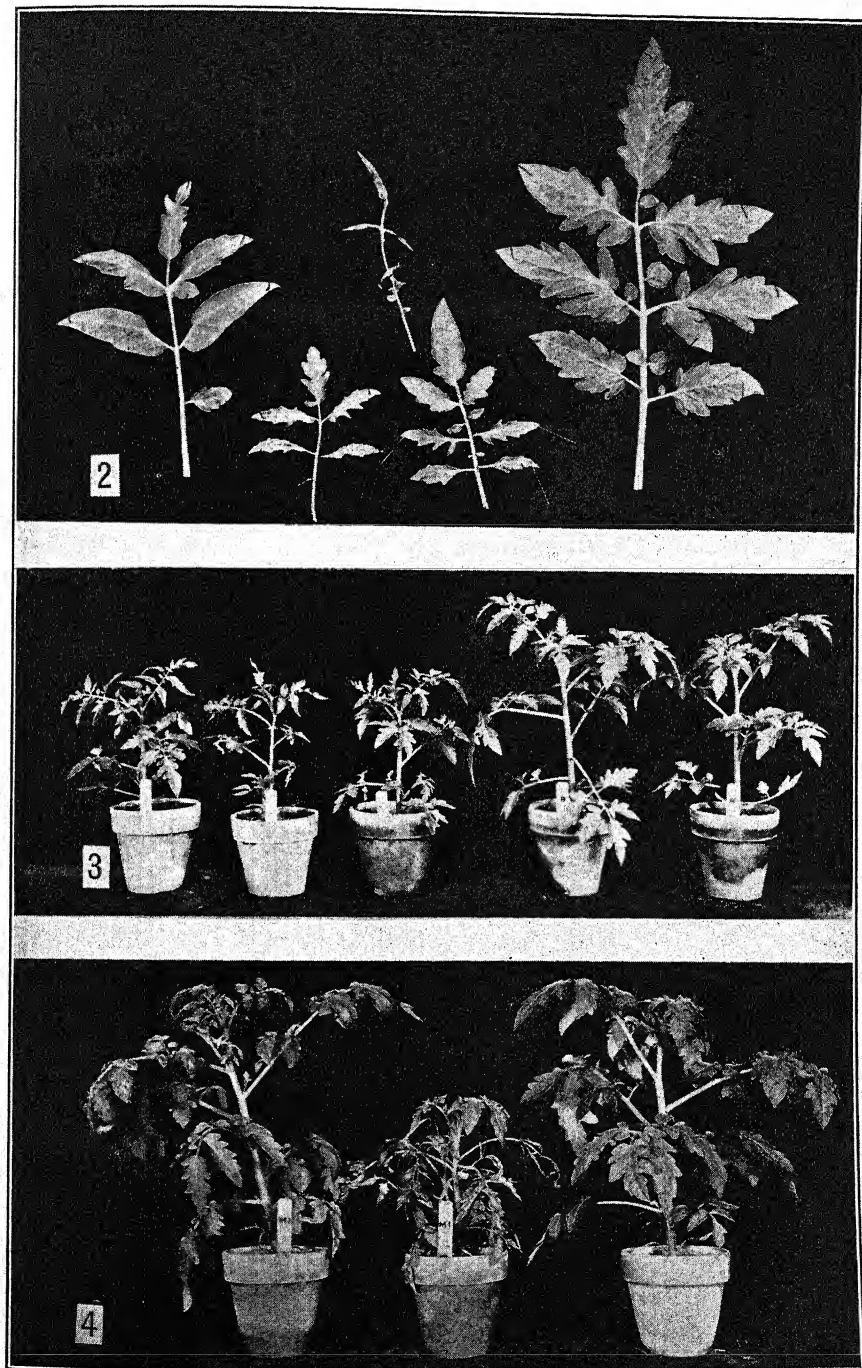




KRAYBILL AND ECKERSON: TOMATO MOSAIC









## SUMMARY

The mottling principle of tomato mosaic did not pass through the fritted glass filters when colloidal substances were present in the juice, but did pass through in part when the colloids had been partially removed before filtration.

The substance producing fern leaf was separated from the mottling principle of tomato mosaic by filtration through collodion membranes, and by filtration of the colloid-containing juice through fritted glass filters.

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YONKERS, NEW YORK

## EXPLANATION OF PLATES

## PLATE LXIII

FIG. 1. Leaves from plants in experiment 5. Control at left; fern leaf at center, inoculated with virus 1A; mottled leaf at right, inoculated with virus 5A. 1A is filtrate and 5A is residue from the same juice. Inoculated April 9, 12, 15; photographed April 27.

## PLATE LXIV

FIG. 2. Leaves from plants in experiment 7. Control at right; 3 fern leaves at center, inoculated with virus 1A; fern leaf at left inoculated with virus 1L. Inoculated May 12, 14; photographed June 6.

FIG. 3. Plants from experiment 5. Controls at right; 3 fern-leaf plants at left inoculated with virus 3A (1A filtered through collodion). Inoculated April 9, 12, 15; photographed April 27.

FIG. 4. Plants from experiment 7. Control at right; mottled plant at left, inoculated with virus 1M; center plant both mottled and fern leaf, inoculated with virus 1M. Inoculated May 12, 14; photographed June 5.

1. 4  
2. 4  
3. 4  
4. 4  
5. 4

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## A STABLE COLORIMETRIC STANDARD FOR CHLOROPHYLL DETERMINATIONS

JOHN D. GUTHRIE

(Received for publication November 25, 1927)<sup>1</sup>

The usual standard used in the colorimetric determination of chlorophyll is a solution resulting from the saponification of the pigment itself. Such a standard has several disadvantages, among which are that: (1) it is unstable; (2) standards prepared from time to time may differ slightly; (3) pure chlorophyll is needed for its preparation, and this is not available to many workers; and (4) those having chlorophyll preparations have no assurance that theirs is of the same purity as those of others.

Owing to its ease of exact duplication and its stability the standard described here does not have these disadvantages. Those desiring to determine chlorophyll but not having the pure pigment should find it especially valuable.

The method for which this standard was devised is essentially that of Willstätter and Stoll<sup>2</sup> for total chlorophyll. For a standard in this method pure chlorophyll is dissolved in ether, saponified with potassium hydroxid, extracted from the ether with water, and made to volume. For details of the method the original should be consulted.

In searching for a colored solution to replace the standard prepared from chlorophyll itself the idea of using a dye mixture was abandoned, since many dyes are unstable or cannot be obtained in a known state of purity. It was found that a solution consisting of a mixture of copper sulphate, potassium dichromate, and ammonium hydroxid could be used. Solutions of these substances of known concentration can easily be made.

The following solutions were used:

1. Copper sulphate,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 10 grams per liter. Crystals covered with the white powder of the less hydrated form should be avoided. If possible, the purity of the salt should be checked.

2. Potassium dichromate,  $\text{K}_2\text{Cr}_2\text{O}_7$ , 20 grams per liter.

3. Ammonium hydroxid, twice normal.

To make the standard, measure accurately 28.5 cc. of the copper sulphate, 50 cc. of the potassium dichromate, and 10 cc. of the ammonium hydroxid into a 100 cc. volumetric flask; make to volume and mix. When the solution was placed in one cup of the colorimeter and the instrument set at

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

<sup>2</sup> Untersuchungen über Chlorophyll, 78-83. Berlin, 1913.

20, 30, or 40 mm. it proved to be colorimetrically equivalent to a solution obtained by saponifying 85 mg. of chlorophyll and making up to one liter.

The green weight percentages of chlorophyll, determined on several leaf samples with both the usual chlorophyll standard and the new standard, are shown as follows:

With Chlorophyll Standard	With New Standard
.305.....	.302
.318.....	.322
.230.....	.230
.187.....	.189
.335.....	.333
.250.....	.248

The author hopes that workers having pure chlorophyll at hand will check the value given as the chlorophyll equivalent of the new standard here described.

DEPARTMENT OF AGRICULTURAL CHEMISTRY,  
OHIO STATE UNIVERSITY

## FURTHER STUDIES IN THE RING-SPOT DISEASE OF TOBACCO

C. N. PRIODE

(Received for publication November 25, 1927)<sup>1</sup>

In a recent paper<sup>2</sup> some of the symptoms of the ring-spot disease of tobacco were described and pictured. A careful study of these symptoms led to the belief that ring spot belongs in the virus disease group of plant maladies. The object of the present paper is to report the results of some further studies on the disease. Special attention has been given to symptoms, host range, and means of transmission. Studies have also been made on the keeping qualities of the infectious agent in juices stored at different temperatures.

Although ring spot frequently produces chlorosis which causes mottling, it is essentially an infectious necrosis. On tobacco it attacks leaf tissues only. No visible symptoms ever appear on stems or on large leaf veins. It is typically a leaf-spot disease. Chlorotic and necrotic spots occur in the form of small circular areas, rings with green centers, concentric circles, or wavy lines which follow along the leaf mid-ribs and leaf veins. The tissues composing the spots commonly die. In some cases, however, they live indefinitely but are more or less chlorotic.

Ring spot is a systemic disease. Lesions usually appear on all or most of the leaves above the point of inoculation. It differs from most virus diseases, however, in that the first symptoms appear at or near the point where a leaf is inoculated. Systemic infection occurs a few days after the first symptoms of local infection appear.

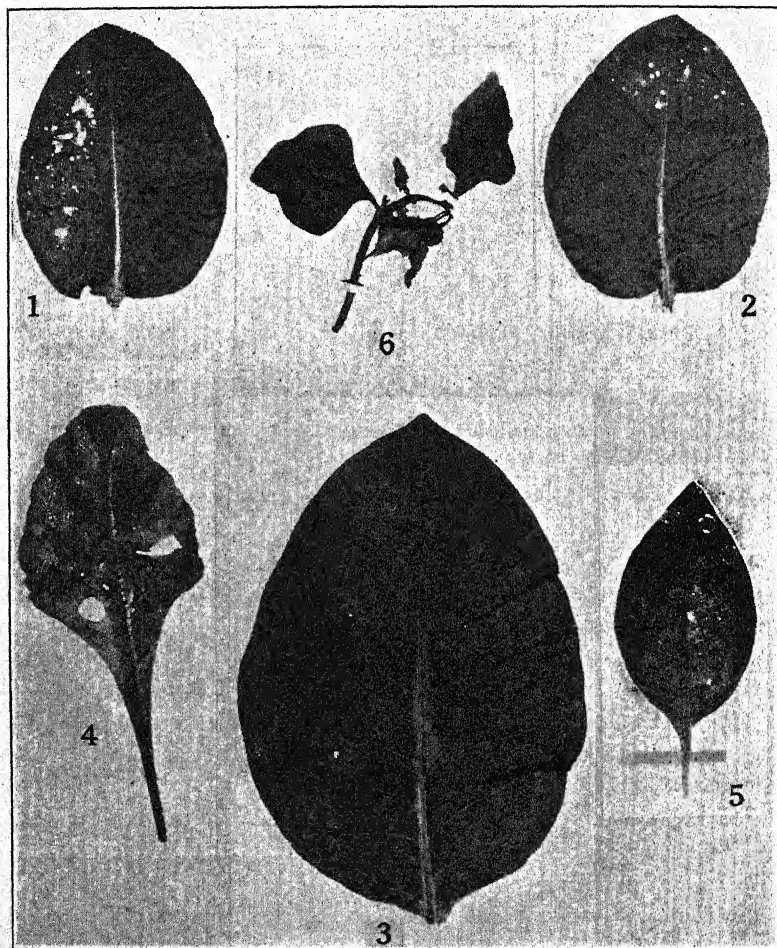
Ring-spot disease has been produced repeatedly on definite portions of leaves by applying inoculum to those portions. In one experiment thirty plants were inoculated. One leaf on each of ten plants was inoculated by applying plant juice containing the infectious agent to portions of the blade on one side of the mid-rib. Within four days rings developed on the inoculated portions. Uninoculated portions of these leaves remained healthy, as shown in text figure 1. Ten of the plants were inoculated by applying infectious juice to the basal portions of a leaf on each plant. After four days rings developed on the inoculated areas but did not appear on other parts of the inoculated leaves. The other ten plants were inoculated by applying infectious juice to the tip portions of their leaves. Here

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

<sup>2</sup> Fromme, F. D., S. A. Wingard, and C. N. Priode. Ringspot of tobacco; an infectious disease of unknown cause. *Phytopath.* 17: 321-328. 1927.



again rings appeared on the areas inoculated but did not appear on uninoculated parts, as is shown in text figure 2. Five days after the development of local symptoms, rings appeared on the young leaves of all of the thirty plants. In every case local infection was followed by systemic infection. In other experiments a few instances were observed where systemic infection failed to follow local infection. In such cases the disease occurs in inoculated tissues only. If inoculations are made into stem tissues systemic infection occurs without the production of local symptoms.



TEXT FIG. 1. Rings produced on half of tobacco leaf to which inoculum was applied. TEXT FIG. 2. Rings produced on tip portion of tobacco leaf where inoculum was applied. TEXT FIG. 3. Tobacco leaf showing rings formed around needle-puncture inoculations. Two uninoculated needle-puncture wounds are shown on the side opposite the ring spots. TEXT FIG. 4. Ring spot on a beet leaf. TEXT FIG. 5. Ring spot on a leaf of *Phytolacca decandra*. TEXT FIG. 6. Wilt produced by ring spot on New Zealand spinach.

Other experiments were performed which gave further proof of the ability of the disease to produce local infection at the point of inoculation. The pointed end of a needle was wrapped with a small bit of absorbent cotton. The cotton was then saturated with infectious juice. One hundred and twenty needle-prick inoculations were made into the leaves of healthy plants. Enough pressure was used to force some of the juice from the cotton into each of the needle-prick wounds. Similar uninoculated needle-prick wounds in the same leaves served as checks. Six days after the inoculations were made, rings had formed around thirty-six of the one hundred and twenty needle-puncture inoculations. No infections resulted apparently from the other eighty-four inoculations or from the check needle-puncture wounds. Text figure 3 shows some of the rings and their relation to the needle wounds. In most instances the rings formed concentrically around the needle punctures. In some cases, however, they developed eccentrically. Each successful inoculation produced a single ring spot with its center at or near the point where the needle jab was made.

Although ring spot occurs abundantly on tobacco in the field it has never been observed on other species of plants. The ease of mechanical transmission of ring spot from diseased to healthy tobacco plants suggested the possibility that it might be transmitted to other species. Accordingly about 20 different species, including potato, tomato, pepper, and eggplant, were inoculated with juice from diseased tobacco plants. The disease was successfully transmitted to only four of the plants subjected to the test. The plants found to be susceptible are the beet (*Beta vulgaris* L.), pokeweed (*Phytolacca decandra* L.), petunia (*Petunia hybrida*) and New Zealand spinach (*Tetragonia expansa* Murr.). Beet and pokeweed plants developed typical rings on the leaves to which the inoculum was applied. No symptoms appeared elsewhere on the plants. Systemic infection was not obtained. A diseased beet leaf is shown in text figure 4 and a diseased pokeweed leaf in text figure 5. Petunia plants proved to be very susceptible to the disease. Symptoms on petunia were similar to those on tobacco. Ring spots appeared first on the portions of leaves to which inoculum was applied. Three to four days after the appearance of local symptoms systemic infection appeared on the young leaves at the tips of the branches. Young petunia leaves show chlorosis rather than necrosis during early stages in the development of the disease. The chlorotic areas consist of wavy lines and rings. After a few days most of the affected tissues die and collapse but some of the chlorotic rings remain alive indefinitely. Ring spots similar to those observed on tobacco leaves were produced on full-grown petunia leaves by means of needle-prick inoculations. In one experiment rings developed around twenty-seven of forty-five needle-prick wound inoculations.

The symptoms of ring spot on New Zealand spinach are similar to those on tobacco and petunia. Unlike any of the other hosts, however, New Zealand spinach shows stem symptoms. These appear first as small,

discolored, depressed streaks or elliptical ring spots near the points at which diseased leaves are borne. As the disease develops the streaks and rings become more pronounced and extend to all upper portions of diseased stems. Necrosis in the stem tissues of this plant is so severe that the tips of some branches wilt and die, as is shown in text figure 6. Microscopic examination of free-hand sections of diseased stems shows necrosis and blackening of affected tissues. No trace of bacteria or fungi could be found in the stem lesions. Young tobacco plants inoculated with juice from diseased New Zealand spinach stems developed typical ring-spot symptoms after five days.

It is well known that the infective principle of the common tobacco mosaic is retained in dried leaves for long periods of time. The infective agent of ring spot apparently loses its virulence after a short period of drying in diseased leaves. Tobacco leaves affected with ring spot were harvested and divided into two portions. One lot was hung up in a greenhouse to dry; the other was allowed to dry in a laboratory where the light was less intense. After one month the leaves of each lot were ground to a fine powder. The powder was placed in a small amount of water in each of two flasks. After thirty minutes of soaking, material from each lot was used to inoculate eight young tobacco plants. All of the plants remained healthy. Similar experiments with juice expressed from diseased plants were undertaken. It was found that the causal agent in ring-spot juice quickly loses its virulence when stored at or above room temperature. At lower temperatures the virus retains its virulence for a much longer period of time. On April 1, 1927, six flasks, containing a small quantity of juice from diseased leaves, were stored at the following temperatures:  $-5^{\circ}\text{C.}$ ,  $0^{\circ}\text{C.}$ ,  $5^{\circ}\text{C.}$ ,  $10^{\circ}\text{C.}$ ,  $15^{\circ}\text{C.}$ , and  $20^{\circ}\text{C.}$  Young tobacco plants were inoculated with juice from each of the flasks one day after they were stored and at different intervals thereafter as shown in table 1. It will be seen that the only sample of virus that retained its virulence over the period of 85 days during which the experiment was in progress was that held at  $-5^{\circ}\text{C.}$  The sample held at  $0^{\circ}\text{C.}$  lost its virulence after about three weeks; that held at  $20^{\circ}\text{C.}$  after one day; that held at  $15^{\circ}\text{C.}$  after four days; that held at  $10^{\circ}\text{C.}$  after 12 days, and that held at  $5^{\circ}\text{C.}$  after twenty days. The experiment shows that the length of time during which the virus retains its virulence in expressed juice varies inversely with the temperature at which the juice is stored.

The above experiments suggest that ring spot is not carried over winter in dead tissues. It was thought that the disease might be transmitted through seeds. In order to test this, six hundred tobacco plants were grown to maturity from seeds taken from badly diseased plants. All of these plants remained healthy, indicating that the disease is not readily transmitted through seeds.

Many of the characteristics of ring spot indicate that it belongs in the

TABLE I. *Effect of Temperature on the Inactivation of the Ring-spot Virus When Stored*

No. Days Stored	Temperature at Which Juice Was Stored											
	- 5° C.		0° C.		5° C.		10° C.		15° C.		20° C.	
	No. Plants Inocu- lated	No. Plants In- fected	No. Plants Inocu- lated	No. Plants In- fected	No. Plants Inocu- lated	No. Plants In- fected	No. Plants Inocu- lated	No. Plants In- fected	No. Plants Inocu- lated	No. Plants In- fected	No. Plants Inocu- lated	No. Plants In- fected
1	5	5	5	3	5	3	5	3	5	1	5	0
3	5	5	5	4	5	4	5	3	5	0	5	0
7	4	3	4	4	4	3	4	2	4	0	4	0
11	4	3	4	3	4	2	4	0	4	0		
15	4	4	4	3	4	2	4	0	4	0		
17	4	4	4	1	4	1	4	0				
19	5	5	5	1	5	0						
21	5	4	5	0	5	0						
23	5	4	5	0								
29	4	4										
35	4	4										
44	4	4										
60	4	3										
75	4	4										
85	4	4										

virus disease group. Since the agents causing most of these diseases are filter-passers, an effort was made to determine whether or not the virus of ring spot is filterable. A portion of juice extracted from diseased leaves by grinding and pressing was passed through a Berkfeldt filter of grade "N" and inoculated into each of ten healthy young tobacco plants. At the same time an unfiltered portion of the same juice was inoculated into four similar healthy young tobacco plants. After four days the plants inoculated with unfiltered juice became diseased. The ten plants inoculated with filtered juice remained healthy. This experiment was repeated with another sample of virus-bearing juice. The disease was not obtained by inoculating with filtered juice. Whether the agent causing ring spot will pass filters of coarser grade than "N" has not been determined. Although the infective agent did not pass the filters used, ring spot is nevertheless believed to belong in the virus group of plant diseases.

#### SUMMARY

1. Ring spot produces both localized and systemic infections. Local symptoms appear 3-5 days after inoculation. Systemic infection develops a few days later.
2. Rings were produced around needle-prick inoculations on both tobacco and petunia plants.
3. Ring-spot infection was produced on four hosts other than tobacco: beet, pokeweed, petunia, and New Zealand spinach. Unlike the other hosts, New Zealand spinach developed symptoms on the stems.

4. No infection was produced from dried diseased material.
5. Juice stored at  $-5^{\circ}\text{C}$ . produced infection when applied to plants 85 days after extraction. When juice is stored at higher temperatures, virulence is lost very rapidly.
6. The infectious principle in juice from ring-spot tobacco plants does not pass a Berkfeldt filter of grade "N."

The writer is indebted to Dr. L. O. Kunkel for valuable suggestions and advice during the progress of this work, and for a careful reading and criticism of the manuscript.

TROPICAL PLANT RESEARCH FOUNDATION,  
CENTRAL BARAGUA, BARAGUA,  
PROVINCIA DE CAMAGUEY, CUBA



## MULTIPLICATION OF THE VIRUS OF TOBACCO MOSAIC IN DETACHED LEAVES

HELEN A. PURDY

(Received for publication November 25, 1927)<sup>1</sup>

### INTRODUCTION

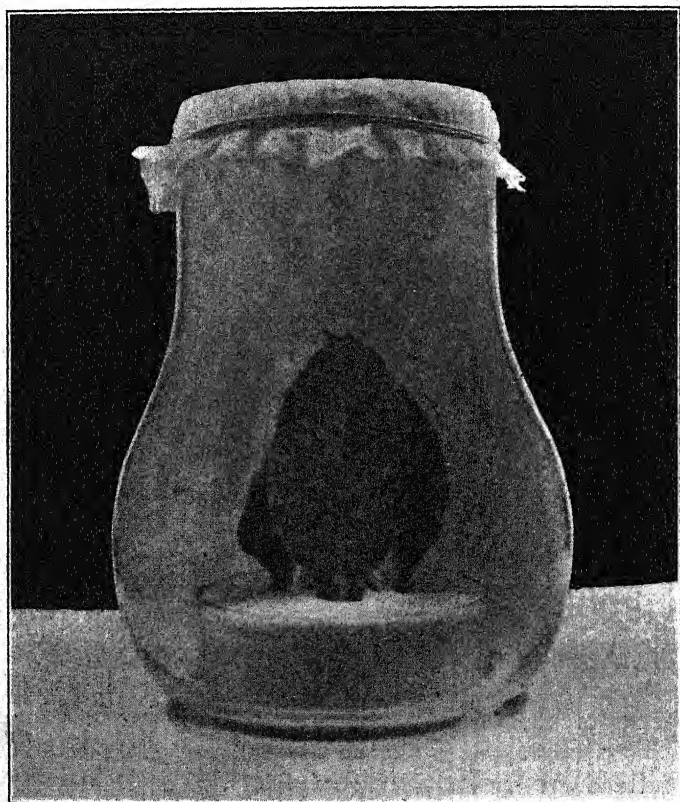
When a tobacco plant takes mosaic disease, the first macroscopic evidence of infection is a clearing of the veins followed by a mottling of the youngest leaves of the shoot. Any immature leaf younger than the one inoculated may exhibit this symptom, but a leaf that is fully formed at the time the virus is introduced into the plant never becomes mottled. The correlation existing between age of leaves and the most characteristic external manifestation of mosaic disease suggests that multiplication of the virus may require the presence of meristematic tissue. But as Allard (1) has demonstrated, all leaves of an affected plant, irrespective of mottling, contain infectious sap. If we assume that the virus is capable of multiplication only in formative tissues, there must be a subsequent distribution from these localized areas.

It seemed likely that some pertinent information might be obtained through an effort to multiply the active principle of mosaic disease in leaves detached from tobacco plants just prior to inoculation. Accordingly, virus was introduced into a number of detached leaves and, after a suitable incubation period, intracellular bodies were observed similar to those described in mosaic tobacco by Iwanowski (5), Goldstein (3, 4), Rawlins and Johnson (7), and others. The presence of these cell inclusions was interpreted as evidence of infection. Moreover, the sap expressed from these leaves, after dilution far beyond the point required to inactivate the virus originally introduced (2), produced one-hundred-percent mosaic disease when inoculated into healthy tobacco plants. The results of these preliminary tests plainly indicated that a multiplication of the virus takes place in the detached leaves. To confirm this finding, serial transmission experiments were undertaken (6) whereby the virus introduced into the first detached leaf of each series would soon become inactivated by high dilution in the course of transmission from leaf to leaf. Therefore, unless the virus multiplied, sap from detached leaves receiving the original inoculum only in high dilution would not be infectious.

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

## METHODS

Leaves varying in length from 9.9 to 21.4 cm. were detached from healthy tobacco plants and inoculated by rubbing 0.01 or 0.02 cc. of undiluted sap from mosaic tobacco leaves into a scarified area at the tip of each leaf. Following insertion in moist quartz sand, the inoculated leaves were placed under lantern globes and kept in a laboratory for a period of incubation varying from 7 to 24 days. The lantern globe was used to exclude insects and also to produce a moist atmosphere. Text figure 1 shows the way in which leaves were incubated while the experiment was in progress.



TEXT FIG. 1. Method of incubating leaves.

The sand was moistened daily with distilled water. In order to ascertain whether or not any appreciable growth takes place in the detached leaves during incubation, a number of leaves were measured from base to tip along the mid-rib at the time of inoculation with virus and again at the end of the period of incubation. The leaves were then examined for the presence of cell inclusions commonly associated with tobacco mosaic. A small piece of epidermal tissue was stripped from the back of a mid-rib or vein and mounted

in a 0.5-percent aqueous solution of iodine green. After a few moments, the host nuclei appeared turquoise blue while the intracellular bodies became pinkish.<sup>2</sup> No attempt was made to find the inclusions in all of the detached leaves studied. They were found in enough instances to show that they are commonly produced in affected detached leaves. At the end of the incubation period, the sap was expressed from each detached leaf and 1 cc. of distilled water was added in every case. Either 0.01 or 0.02 cc. of this diluted sap was then inoculated into a second detached leaf. The entire process was repeated through eleven series, in six of which ten leaves were inoculated successively. In order to increase the possibility of transmission of the virus in every series, several detached leaves were inoculated with sap from the same leaf of a given series. By this method, it was possible to estimate the approximate amount of virus introduced into each detached leaf of every series, assuming that no multiplication had occurred.

The infectiousness of the sap expressed from the detached leaves was determined in every case by its ability to induce mosaic disease in tobacco or tomato plants.

The virus employed for inoculating the first leaves of the different series was obtained from two sources, but the results in each case were similar. Tobacco leaves affected with a common field mosaic, kindly supplied by Doctor James Johnson, were taken as the source of virus for seven of the series, while mosaic leaves from a tobacco plant growing in a greenhouse at the Boyce Thompson Institute were employed for the four remaining series. Throughout the experiments every precaution was taken to avoid accidental transmission of virus. Additional leaves were taken as controls from all plants used as a source of detached leaves. Sap from a healthy plant was substituted for virus as a source of inoculum, otherwise the experiments described above for serial transmission of the virus were carefully duplicated. It is evident that the control leaves would serve as a check in the event that leaves were detached from a plant affected with mosaic disease in its incipient stages, for in such a case sap from the control leaf would induce infection in the inoculated control plants.

The total number of leaves included in the serial transmission experiments in which virus was used is given in table 2. Of the 171 leaves inoculated, the sap from 146 caused mosaic disease in tobacco and tomato plants, indicating a successful transmission of virus in the majority of cases.

The dilution of the original inoculum introduced into every detached leaf of each series was computed. The number of leaves inoculated with a given dilution is reported in table 3 with the results obtained from testing the infectivity of the sap expressed from each of these leaves. Of 142 detached leaves inoculated with dilutions of the original inoculum exceeding

<sup>2</sup> This contrast staining reaction is not permanent but lasts only a few hours. This stain was suggested to me by Doctor Francis O. Holmes as a useful means of differentiation between the host nucleus and the intracellular body.



$2 \times 10^{-5}$ , 120 contained sap capable of inducing mosaic disease in tobacco and tomato plants.

### RESULTS

The results obtained from attempts to transmit virus through eleven individual series of detached leaves are recorded in table 1.

TABLE 1. *Transmission of the Virus in Individual Series*

Detached Leaf Inoculated	No. of Individual Series Included	No. of Leaves Containing:	
		Infectious Sap	Non-infectious Sap
1st leaf of series.....	11	10	1
2d " " ".....	10	9	1
3d " " ".....	9*	7	1
4th " " ".....	7	7	0
5th " " ".....	7	7	0
6th " " ".....	7	7	0
7th " " ".....	7	6	1
8th " " ".....	6	6	0
9th " " ".....	6	6	0
10th " " ".....	6	6	0
Total.....	75	71	4

\* Sap from the corresponding control leaf in one case also proved infectious; therefore the series was discontinued and the results excluded.

TABLE 2. *Results of Inoculations of All Leaves of Each Series*

Detached Leaf Inoculated	No. Detached Leaves Inoculated	No. of Leaves Containing:	
		Infectious Sap	Non-infectious Sap
1st leaf of series.....	11	10	1
2d " " ".....	10	9	1
3d " " ".....	9*	7	1
4th " " ".....	10†	10	0
5th " " ".....	24	22	2
6th " " ".....	25	22	3
7th " " ".....	18	17	1
8th " " ".....	17	12	5
9th " " ".....	18	16	2
10th " " ".....	30	21	9
Total.....	171	146	25

\* Cf. footnote to table 1.

† In order to increase the possibility of transmission of the virus in every series, several detached leaves were inoculated with sap from the same leaf of a given series.

Forty-one detached leaves in which virus multiplied showed an average increase in length during incubation of only 1.58 cm., or 9 percent of the average length of the leaves. In one case, a leaf increased 14 percent of its length, while the minimum elongation observed was about 0.6 percent.

TABLE 3. *Results of Inoculations of Detached Leaves With Various Dilutions of Virus*

Dilution of Original Inoculum	No. Detached Leaves Inoculated *	No. of Leaves Containing:	
		Infectious Sap	Non-infectious Sap
Undiluted 0.01 cc.....	4	4	0
Undiluted 0.02 cc.....	7	6	1
2 x 10 <sup>-2</sup> .....	5	5	0
1 x 10 <sup>-2</sup> .....	5	4	1
4 x 10 <sup>-4</sup> .....	4	3	1
2 x 10 <sup>-4</sup> .....	3	3	0
1 x 10 <sup>-4</sup> .....	1	1	0
8 x 10 <sup>-6</sup> .....	3	3	0
4 x 10 <sup>-6</sup> .....	4	4	0
2 x 10 <sup>-6</sup> .....	3	3	0
16 x 10 <sup>-8</sup> .....	7	7	0
8 x 10 <sup>-8</sup> .....	10	8	2
4 x 10 <sup>-8</sup> .....	7	7	0
32 x 10 <sup>-10</sup> .....	7	6	1
16 x 10 <sup>-10</sup> .....	11	9	2
8 x 10 <sup>-10</sup> .....	7	7	0
64 x 10 <sup>-12</sup> .....	2	2	0
32 x 10 <sup>-12</sup> .....	11	10	1
16 x 10 <sup>-12</sup> .....	3	3	0
8 x 10 <sup>-12</sup> .....	2	2	0
64 x 10 <sup>-14</sup> .....	6	3	3
32 x 10 <sup>-14</sup> .....	6	4	2
16 x 10 <sup>-14</sup> .....	5	5	0
128 x 10 <sup>-16</sup> .....	7	7	0
64 x 10 <sup>-16</sup> .....	4	3	1
32 x 10 <sup>-16</sup> .....	7	6	1
256 x 10 <sup>-18</sup> .....	9	9	0
128 x 10 <sup>-18</sup> .....	9	7	2
64 x 10 <sup>-18</sup> .....	12	5	7
Total.....	171	146	25

\* Cf. footnote to table 1.

No mottling of the detached leaves ever occurred. Those in which virus multiplied were macroscopically indistinguishable from the corresponding control leaves.

#### DISCUSSION

There were doubtless some dividing cells present in the detached leaves during the incubation period. Probably after detachment from the plant, meristematic tissues are produced in some of the leaves. It was observed that when detached leaves were allowed to remain in the quartz sand for four or five weeks, they developed roots. But considering the brief incubation period of one week, which was adequate for multiplication of the virus, active growth of the detached leaves was reduced to a minimum.

It will be noted that the dilution of the 0.01 or 0.02 cc. of inoculum introduced into the first leaf of each series attained through serial transmission is estimated quite conservatively, since no account is taken of the sap present in the inoculated leaf, which varied in amount from approximately 0.5 to 1.5 cc. Also it was assumed that all of the inoculum intro-

duced was recovered from each macerated leaf, whereas only part of the sap actually was expressed. Consequently, the dilutions of original inoculum recorded in table 3 as capable of inducing mosaic disease in plants after incubation in a detached leaf are considerably underestimated.

#### SUMMARY

Leaves were detached from healthy tobacco plants and inoculated with the virus of tobacco mosaic. After a suitable incubation period, characteristic intracellular bodies were found in many of the detached leaves, although no mottling nor other macroscopic symptom of disease was apparent. The presence of these cell inclusions was interpreted as evidence of infection.

It was then shown by serial transmission experiments that the causal agent of tobacco mosaic multiplies in the detached leaves. The presence or absence of virus in each detached leaf was determined by the ability of the extracted sap to induce mosaic disease in healthy tobacco or tomato plants.

The author wishes to express her gratitude to Doctor L. O. Kunkel for suggestions during the course of these experiments and for a critical review of the manuscript.

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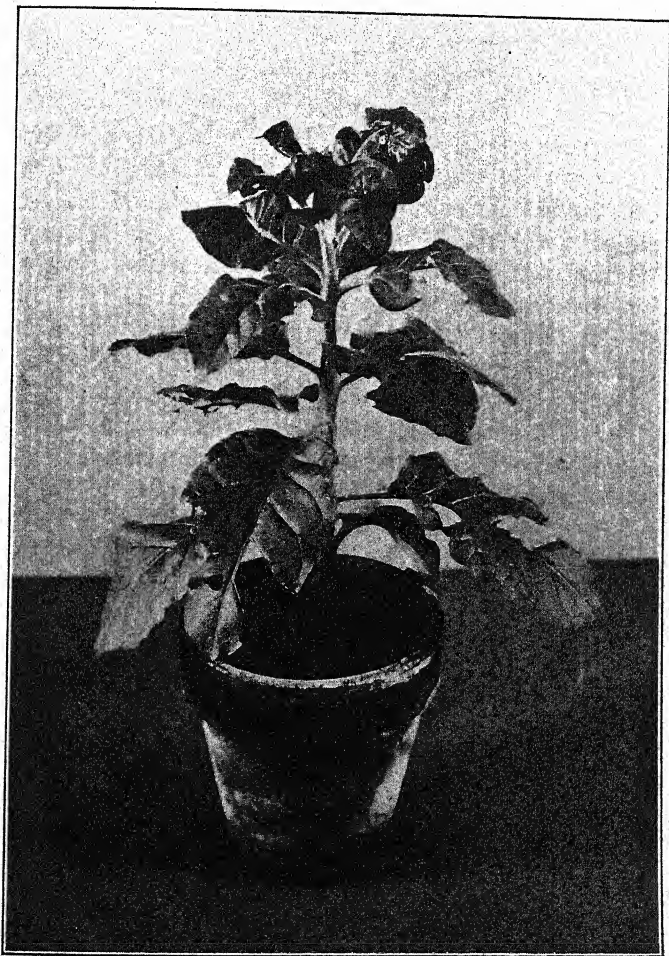
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## THE IMPROBABILITY OF TOBACCO MOSAIC TRANSMISSION BY SLUGS

HELEN A. PURDY

(Received for publication November 25, 1927)<sup>1</sup>

During an infestation of slugs in greenhouses among tobacco and tomato plants used for the study of mosaic disease, the question arose as to whether or not these pests could transmit the disease. Experiments were carried out in which slugs that had fed upon tobacco and tomato plants affected with mosaic disease were transferred to healthy tobacco and tomato plants and allowed to feed heavily upon them.



TEXT FIG. 1. Healthy tobacco plant upon which infested slugs have fed.

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

The slugs were confined under bell jars upon diseased plants for two to fourteen days. They were then transferred to healthy plants for periods varying from one to twenty-seven days. Two species of slugs were used in these experiments, *Limax maximus* and *L. agrestis*, kindly identified by Doctor Roy Miner of the American Museum of Natural History. The slugs were not placed directly upon the leaves of a plant but were allowed to crawl up to the plant from the base of the pot, as they would pass naturally from one plant to another in the greenhouse experiments. From three to eight slugs were transferred from a diseased to a single healthy plant. Text figure 1 shows a healthy tobacco plant upon which slugs from a diseased plant were allowed to feed. The extent to which the slugs fed is shown. The distortion at the tip of the shoot is the result of crowding in a lantern-globe cage.

Twenty-three attempts were made to transmit mosaic disease through slugs by transferring them after feeding upon diseased plants directly to healthy ones. *Limax maximus* was used in all but three of the cases. In eleven of the twenty-three attempts the slugs were transferred successively through series comprising two to five healthy plants, allowing one or two days for feeding upon each healthy plant. A total of fifty-one plants were tested for transmission of tobacco mosaic by this method and not a single case of infection resulted. However, when slugs that had fed upon mosaic material were macerated, they were found to contain bits of green plant tissue which, when inoculated into healthy plants, readily produced infection.

The results of these experiments indicate that transmission of mosaic disease among tobacco and tomato plants in greenhouses through the agency of slugs may be regarded as very improbable.



## CHEMICAL TREATMENTS FOR SHORTENING THE REST PERIOD OF POT-GROWN WOODY PLANTS

F. E. DENNY AND ERNEST N. STANTON

(Received for publication February 18, 1928)<sup>1</sup>

When woody plants such as lilac and flowering almond are grown in pots in the summer and are brought into the greenhouse in the succeeding fall in order to force them into bloom, it is found that a considerable period of time elapses before the buds open. Most species of woody plants exhibit this dormant period, the length of which varies greatly with the kind of plant and with the conditions (especially temperature) to which the plant is exposed just previous to transference to the warm house.

Johannsen (5) showed that treatment with chemical vapors such as ether and chloroform would break this dormant period and permit prompt growth of buds. Howard (4) in a series of experiments confirmed and extended this work and applied the treatments to a number of species. Stuart (6) showed that not only ether and chloroform but a number of other chemicals including ethyl bromid, ethyl iodid, carbon tetrachlorid, etc., could be successfully used. Our work extends these observations and adds to the list a number of other chemicals not previously used, two of which give promise of being specially valuable for practical work.

Because of the success that was obtained with certain chemical vapors in the treatment of dormant potato tubers (Denny 2, 3), tests of these chemicals and others of a similar nature were made. The experiments were carried out mainly with common lilac (*Syringa vulgaris* L.) and its variety "Charles X," flowering almond (*Prunus triloba* Lindl.), Bechtel's flowering crabapple (*Pyrus ioensis* Bailey), *Azalea nudiflora* L., *Deutzia gracilis* Sieb. and Zucc., and snowball (*Viburnum tomentosum* Thunb.).

Favorable results were obtained with all the species tested except snowball. The vapors of such chemicals as ethylene chlorhydrin, propylene chlorhydrin, ethylene dichlorid, vinyl chlorid, furfural, carbon tetrachlorid, acetylene tetrachlorid, ethyl bromid, and ethyl iodid forced early development of both leaf and flower buds. The gain in the time of budding varied from about two weeks in the case of flowering almond to as much as two months in the case of crabapple.

This paper describes the methods that were used in applying the chemicals, the concentrations and times of exposures that were found to be most favorable, and the responses that were given by the different species.

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

## METHODS

### Preparation of Plants for Treatments

Woody plants were obtained from nurseries in the spring of 1927. The lilacs, crabapples, flowering almonds, and snowballs were about three to four feet high, and the deutzias were about two feet high. The plants were potted into 12-inch clay pots and were buried, pot and all, in the soil, the top of the pot being at the surface of the soil. The plants made only a fair amount of growth during the summer of 1927, their growth being seriously interfered with by attacks of aphids. The flowering almonds formed abundant flower buds, and the deutzias formed a medium number of flower buds, but flower-bud development was sparing or absent in the case of the lilacs and crabapples. After the first frost in the fall (about November 8) the potted plants were removed from the soil and were stored in a basement room at a temperature of about 18° C. The plants, being stored indoors until treatments began, were not exposed to those low temperatures of outdoor conditions which are favorable for the loss of dormancy. Hence the plants remained in their rest period over a longer time, thus affording better opportunities for studying the effects of chemical treatment. The treatments began November 14 and continued until December 24.

### Method of Exposing Plants to Chemical Vapors

Two types of containers were used: (1) A square galvanized iron box, capacity 4,200 liters, with a tight fitting door; an electric fan (of non-sparking type to avoid danger of an explosion) was installed in the box and at the beginning of each treatment the fan was turned on for about an hour in order to distribute the vapors within the enclosure. (2) Several galvanized iron cylinders, 800 liters capacity, equipped with hoisting apparatus; the galvanized iron base was made with a circular slot into which the bottom of the cylinder fitted, the seal being made with moist sand; the chemicals were introduced at the top into a shallow pan, evaporation being spontaneous and not aided by air currents. The temperature during the treatments was about 18° C.

### Procedure After Plants Were Treated

The treated plants were placed in a greenhouse at a temperature of about 70° F. and were examined from day to day for bud development. When the buds opened the pots were fertilized with manure in order to supply the developing buds with proper nutrient supply. In each case the check plants were placed in the greenhouse at the same time, and were fertilized in the same way, whether or not the buds showed any signs of development.

## RESULTS

Flowering Almonds (*Prunus triloba* Lindl.)

Untreated plants of this species began to show development of buds on December 23, and the first blooms opened December 28. The development of blooms in the check plants was irregular. *Prunus* plants treated with vapors of ethylene dichlorid, using 10 cc. of the liquid chemical per 100 liters of air space in the container, came into full bloom on December 13. This result is shown photographically in Plate XIX, *F*. The following treatments also gave early blooming of flowering almond: ethylene (one part of gas by volume to 100 parts of air for three days) (Pl. XIX, *H*); vinyl chlorid (one part of gas by volume to 100 parts of air for one day, and one part to 100 of air for three days); ethylene chlorhydrin,<sup>2</sup> 5 cc. for each 100 liters of air space inside the container for 24 hours (Pl. XIX, *G*). In addition to blooming sooner than the checks, the treated plants bloomed more uniformly, *i.e.* the various flower buds on a plant opened nearly simultaneously.

Common Lilac (*Syringa vulgaris* L.)

Leaf-buds of common lilac, untreated checks, began to unfold January 10, 1928, the development, however, being very irregular, as is shown in Plate XIX, *I*. The checks in fact were not in full leaf at the time the experiments with this species were discontinued on January 23. Common lilac plants treated November 29, 1927, with ethylene chlorhydrin using 5 to 10 cc. to each 100 liters of air space for 24 hours started development of buds in about 10 days after treatment and the plants were in full leaf in about 20 days; ethylene, one part of gas by volume to 1,000 parts of air for three days, caused the swelling of buds in 10 days and, while the subsequent opening of buds was somewhat irregular, a fair amount of leaf growth was attained in 30 days. Ethylene dichlorid, 10 cc. of liquid per 100 liters of space for 24 hours and 2.5 cc. per 100 liters of space for 48 hours, gave good results (Pl. XIX, *J*), as did also the following: propylene chlorhydrin, 8 cc. of the 40-percent solution per 100 liters of space for 48 hours; acetylene tetrachlorid, 0.5 cc. of liquid per 100 liters of space for 24 hours; furfural, 1 cc. of the commercial liquid per 100 liters of air space for 24 hours; vinyl chlorid, one part by volume of the gas to 250–1,000 volumes of air for three days (Pl. XIX, *K*); and carbon tetrachlorid, 2 cc. of the liquid per 100 liters of space for 24 hours.

## Lilac (Variety "Charles X")

The buds of the untreated shrubs did not start to open until January 18, 1928, and the development of buds then occurred irregularly; none of the checks was in full leaf at the end of the experiment. Favorable results

<sup>2</sup> In this paper the ethylene chlorhydrin solution referred to is the 40-percent solution, the concentration ordinarily sold commercially; if the anhydrous 100-percent chemical is available it may be used by making allowance for the dilution.



were obtained with ethylene dichlorid using 10 cc. of the liquid chemical per 100 liters of space in the container for 24 hours. This result is shown in Plate XX, *P*. This plant was treated November 22, 1927, showed budding on December 2, was in full leaf December 12, and was photographed in full bloom December 23. Plate XX, *O*, shows the condition of the check plant on the same day. Other treatments that hastened the development of "Charles X" lilacs were: ethylene chlorhydrin, 5 cc. per 100 liters of space in the container for 24 hours (Pl. XX, *R*), and carbon tetrachlorid, 8 cc. per 100 liters of space for 24 hours (Pl. XX, *Q*).

### *Deutzia gracilis* Sieb. and Zucc.

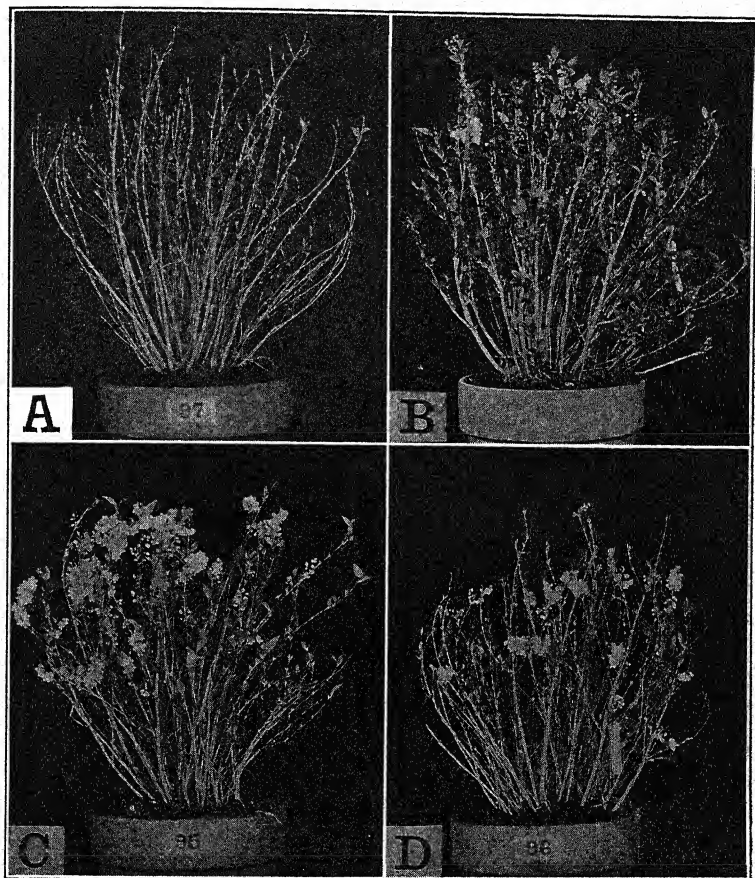
The buds on the untreated deutzias began to grow about December 15, but their rate of development thereafter was very slow and irregular. Some leaves and blooms were formed by the end of the experiment but none of the checks could be regarded as having produced a satisfactory development. The slowness of the rate of budding of the untreated plants may be seen by comparing the check plant in text figure 1, *A*, on December 28, with the check plant in Plate XIX, *L*, on January 16, 1928. *Deutzia gracilis* treated November 16 with ethylene chlorhydrin, 10 cc. per 100 liters of space for 24 hours, showed buds starting November 25 and was in full leaf December 3. This plant is shown in text figure 1, *B*; the photograph, however, illustrating the condition on December 28.

The object of text figure 1 is to show the effect of different concentrations of ethylene chlorhydrin upon the development of leaf buds as contrasted with flower buds. Thus, plant *B* was exposed to vapors of ethylene chlorhydrin using 10 cc. for each 100 liters of space for 24 hours; the concentration for plant *C* was one-fourth that for plant *B*; and for plant *D*, the concentration was one-fourth that for plant *C*. It is seen that the strongest concentration used caused the development of many leaves and few flowers; the weakest concentration, on the other hand, induced the development of flowers but no leaves; the medium concentration produced an intermediate development with respect to leaves and flowers. This effect was observed in another experiment with *Deutzia*, using ethylene dichlorid, but a similar result was not noted with any plant except *Deutzia*. Further experiments are planned to test the possibility of obtaining uniform results in controlling the development of leaf and flower buds in various species by varying the concentration of chemical vapor.

Other treatments that were successful with *Deutzia gracilis* were as follows: ethyl bromid, 5.5 cc. of the liquid chemical per 100 liters of air space for 24 hours (Pl. XIX, *N*); furfural, 12.5 cc. per 100 liters of space for 24 hours (Pl. XIX, *M*); dichlorethylene, 3 cc. per 100 liters of space for 24 hours; propylene chlorhydrin, 12.5 cc. of the 40-percent solution per 100 liters of space for 48 hours; acetaldehyde, 10 cc. per 100 liters of space for 24 hours; ethylene dichlorid, 10 cc. per 100 liters of space for 24 hours; vinyl chlorid 1 : 1,000 for three days.

**Crabapple (Bechtel's Double-flowering, *Pyrus ioensis* Bailey)**

This species was found to be very dormant under the conditions of our experiment. The untreated plants showed no signs of bud development on February 6, 1928. Treatment with ethylene chlorhydrin vapors, 2.5 to



TEXT FIG. 1. Effect of different concentrations of ethylene chlorhydrin upon the relative development of leaves and flowers of *Deutzia gracilis* Sieb. and Zucc. Plant A was the check plant receiving no treatment. Plant B was exposed for 24 hours to vapors of ethylene chlorhydrin, using 10 cc. of the 40-percent solution per 100 liters of space. The concentration for plant C was one-fourth of that for plant B, and the concentration for plant D was one-sixteenth of that for plant B. Note that plant B developed many leaves and few flowers, plant D produced flowers but no leaves, and plant C was intermediate with respect to these features. Plants treated November 16, photographed December 28.

10 cc. per 100 liters of space for 24 hours, caused the development of buds in about 10 days after treatment and gave plants with leaves fully opened in 15 days; the results are shown in Plate XX, T and U. Ethylene dichlorid, 10 cc. per 100 liters of space for 24 hours, induced early develop-

ment of buds and brought the plant into bloom on January 3, 1928. In the case of flowering crabapple, however, only a few plants grew sufficiently well during the summer of 1927 to produce flower buds. Consequently the effect of the treatments upon the blooming of flowering crabapple cannot be determined accurately from these experiments; however, the effect upon the growth of leaf buds was satisfactory. Other treatments that induced development of buds in advance of check plants were: vinyl chlorid, one part of gas to 250 parts of air for three days; propylene chlorhydrin, 12.5 cc. of the 40-percent solution per 100 liters of space for 48 hours (Pl. XX, V); and furfural, 12.5 cc. per liters of space for 24 hours.

### *Azalea nudiflora* L.

Potted plants of this species were received from the Pierson Nursery, Tarrytown, N. Y., on December 23, 1927. Treatments were made December 23 and December 24 and the treated plants with their checks were placed in the greenhouse on December 24. The untreated plants developed flowers irregularly, the first flower opening on January 30, and the best blooming condition was reached on February 6. The treatments with ethylene chlorhydrin are shown in Plate XX, Y and Z. The buds of the plants exposed to vapors of ethylene chlorhydrin, using 6.7 cc. of the 40-percent solution per 100 liters of space for 24 hours, began to open on January 3, 1928 and this plant was in full bloom on January 17. Another plant (not shown in the photograph) was treated with one-third the concentration, that is, 2.2 cc. per 100 liters of space for 24 hours, and this plant bloomed about two days later than plant Y in Plate XX. Plant Z in Plate XX shows the result obtained with the weakest concentration of ethylene chlorhydrin used with *Azalea*, that is, 0.75 cc. per 100 liters of space for 24 hours. This plant bloomed on January 22. Thus, it is seen that the rate of development was influenced by the concentration of the chemical, the weaker concentrations inducing blooming somewhat later.

### Snowball (*Viburnum tomentosum* Thunb.)

The various treatments that were successful in forcing early budding of the other species mentioned in this paper were not effective with *Viburnum tomentosum*. This species seems to require a different treatment. For the next series of experiments the plants will be given a preliminary exposure to low temperatures before the chemical treatments are started.

## DISCUSSION

### Condition of Plants Previous to Treatments

It was pointed out by Coville (1) that the duration of the dormant period of woody shrubs may be markedly shortened by exposing the plants to low temperatures for certain periods; but the plants in our experiments were not exposed to low temperatures at any time. Probably this explains

why the untreated shrubs retained their dormancy for such a long period, and why the growth which finally resulted was so irregular. The chemical treatments, however, overcame the dormancy even under these unfavorable conditions and permitted a simultaneous opening and uniform development of the buds. In future experiments it is planned to bring the potted plants in from the field at an earlier date, and to expose some of them to low temperatures for a few weeks before the chemical treatments are applied.

### Behavior of Different Species

Of the species tested, the flowering almond plants (*Prunus triloba*) had the shortest dormant period, followed in order by *Deutzia gracilis*, common lilac (*Syringa vulgaris*), "Charles X" lilac, flowering crabapple (*Pyrus ioensis*), and snowball (*Viburnum tomentosum*). The *Asalea nudi-flora* plants were received some weeks after the other species and since they had been kept previously under a different set of conditions, it is not possible from the information in this experiment to compare the length of their rest period with that of the other species. After the rest period of the various species was broken the subsequent rate of growth appeared to be satisfactory and normal, except perhaps for the crabapple, in which case the growth was somewhat slow. It would be necessary to make comparisons with non-dormant plants under similar conditions in order to determine whether the treated plants were inferior in this respect. The blooms that were produced by treated plants developed well and appeared to be normal in all cases but one. A flowering almond plant produced flowers noticeably lighter in color than the others but it is not known whether this resulted from the chemical treatment or from a condition that existed in the plant before the treatment was applied.

### Effectiveness of the Different Chemicals

The most favorable results were obtained with ethylene chlorhydrin and ethylene dichlorid. It will require further experiments to show which one of these two is the more effective. They are particularly promising, also, because of their ready availability in commercial quantities at a low price. The dichlorid is lower in price but an important advantage of the chlorhydrin lies in the entire absence of any danger of an explosion with its vapors at ordinary temperature. Among the chemicals that have not been used previously in experiments on hastening the budding of dormant woody plants but which gave good responses in these experiments are propylene chlorhydrin, vinyl chlorid, furfural, and acetylene tetrachlorid. Considerably less favorable results were obtained with iso-propyl formate, methyl salicylate, dichlorethylene, and trichlorethylene. Ethylene was tried in various concentrations and periods of treatment ranging from one part in 100 for three days to one part in 1,000 for one day. It was effective against the less dormant species such as *Prunus triloba* but did not induce



the development of the dormant buds of the more difficult species such as "Charles X" lilac and crabapple. A further disadvantage lies in the longer periods of exposure that are required, three to six days, for ethylene as compared with one day for certain of the other chemicals used. This same objection may also be made against vinyl chlorid, but the greater effectiveness of vinyl chlorid indicated the need of further experiments as to its usefulness.

The following chemicals employed by Stuart (6) in his experiments with potted woody plants were used in these experiments; chloroform, ethyl bromid, ethyl iodid, and carbon tetrachlorid. All of these induced budding considerably in advance of the untreated lots, ethyl bromid giving results somewhat better than the others. Of these four, chloroform gave the least favorable responses. Considerable injury was observed with the ethyl iodid treatments.

### Future Experiments

It is proposed to continue these tests and to extend them to other species and to other chemicals. The variability of the plants has not been given proper consideration yet, and the question whether a group of plants of the same species can be forced into uniform development by a single treatment should have attention. Furthermore, the relation of the stage of dormancy of the plant to the concentration of the chemical and the duration of the exposure needed for good results should be established. The possibility of improving the methods by exposing the plants to a period of chilling previous to the application of the chemical treatments should be tested. Temperature effects during the period of treatment also need consideration.

### SUMMARY

1. Woody plants, flowering size, were planted in the spring in 12-inch pots which were then sunk into the ground. The plants grew in these pots during the summer of 1927.

2. In the autumn of 1927 the potted plants were brought into the laboratory from the field, were placed in metal containers of 800 or 4,200 liters capacity, and were exposed to vapors of various chemicals for the purpose of breaking the rest period of the plants and inducing early development of buds and flowers.

3. The species used were lilac (*Syringa vulgaris* L.); flowering almond (*Prunus triloba* Lindl.); *Deutzia gracilis* Sieb. and Zucc.; crabapple (*Pyrus ioensis* Bailey); *Azalea nudiflora* L., and *Viburnum tomentosum* Thunb. These were brought into leaf or bloom by 24 to 48 hours treatment with the vapors of various chemicals except in the case of the *Viburnum*, which did not respond favorably. The gain in time required for the development of leaves or flowers by treated plants over the checks varied from two weeks in the case of *Prunus* to more than two months in the case of crabapple.

4. The most effective chemicals tried were ethylene dichlorid and ethylene chlorhydrin. The results were sufficiently favorable with propylene chlorhydrin, furfural, vinyl chlorid, and acetylene tetrachlorid to make further tests with them desirable.

5. In the case of *Deutzia gracilis* the relative development of leaves and flowers was modified by the concentration of the chemical used in the treatment.

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#### EXPLANATION OF PLATES

##### PLATE XIX

Top row shows results with flowering almond (*Prunus triloba* Lindl.).

Plant *E* was the check plant, not treated; photographed December 13. Plant *F* was exposed 24 hours to vapors of ethylene dichlorid using 10 cc. of the liquid chemical per 100 liters of space; treated November 22, photographed December 13. Plant *G* was exposed 24 hours to vapors of ethylene chlorhydrin, 5 cc. of the 40-percent solution per 100 liters of space; treated December 9, photographed December 28. Plant *H* was exposed three days to ethylene gas using one part of ethylene to 100 parts of air; treated November 14 to 17, photographed December 23. Note: Only plants *E* and *F* are strictly comparable photographically. Plants *G* and *H* were photographed at later dates, at times when the check plants were further developed, as described in the text.

Middle row shows results with common lilac (*Syringa vulgaris* L.).

Plant *I* was the check plant, not treated; photographed January 16. Plant *J* was exposed 48 hours to vapors of ethylene dichlorid, 2.5 cc. of the liquid chemical per 100 liters of space; treated December 10, photographed January 6. Plant *K* was exposed three days to vinyl chlorid gas, one part of vinyl chlorid to 1,000 parts of air; treated December 12, photographed January 12.

Bottom row shows results with *Deutzia gracilis* Sieb. and Zucc.

Plant *L* was the check plant not treated; photographed January 16. Plant *M* was exposed 24 hours to vapors of furfural, 12.5 cc. of the liquid chemical per 100 liters of space; treated December 6, photographed January 12. Plant *N* was exposed 24 hours to vapors of ethyl bromid, 5.5 cc. of the liquid chemical per 100 liters of space; treated December 1, photographed January 6.

##### PLATE XX

Top row shows results with "Charles X" lilac (*Syringa vulgaris* L.).

Plant *O* was the check plant, not treated; photographed January 16. Plant *P* was exposed 24 hours to vapors of ethylene dichlorid, 10 cc. of the liquid chemical per 100 liters

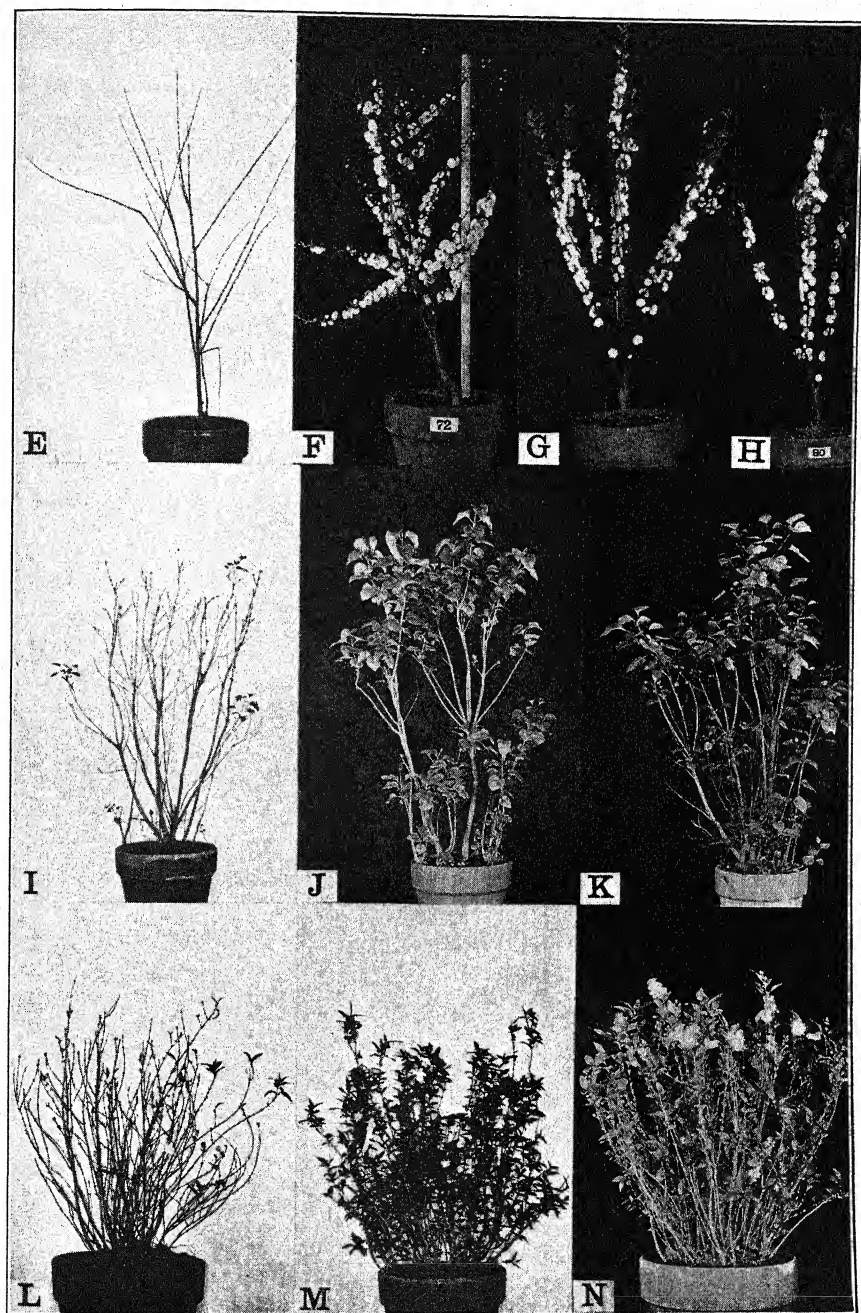
of space; treated November 22, photographed December 23. Plant *Q* was exposed 24 hours to vapors of carbon tetrachlorid, 8 cc. of the liquid chemical per 100 liters of space; treated December 14, photographed January 16. Plant *R* was exposed 24 hours to vapors of ethylene chlorhydrin, 5 cc. of the 40-percent solution per 100 liters of space; treated December 9, photographed January 16.

Middle row shows results with Bechtel's flowering crabapple (*Pyrus ioensis* Bailey).

Plant *S* was the check plant, not treated; photographed January 16. Plant *T* was exposed 24 hours to vapors of ethylene chlorhydrin, 5 cc. of the 40-percent solution per 100 liters of space; treated December 9, photographed January 16. Plant *U* was exposed 24 hours to vapors of ethylene chlorhydrin, 2.5 cc. of the 40-percent solution per 100 liters of space; treated November 29, photographed January 16. Plant *V* was exposed 48 hours to vapors of propylene chlorhydrin, 12.5 cc. of the 40-percent solution per 100 liters of space; treated December 3, photographed January 16.

Bottom row shows the results with *Azalea nudiflora* L.

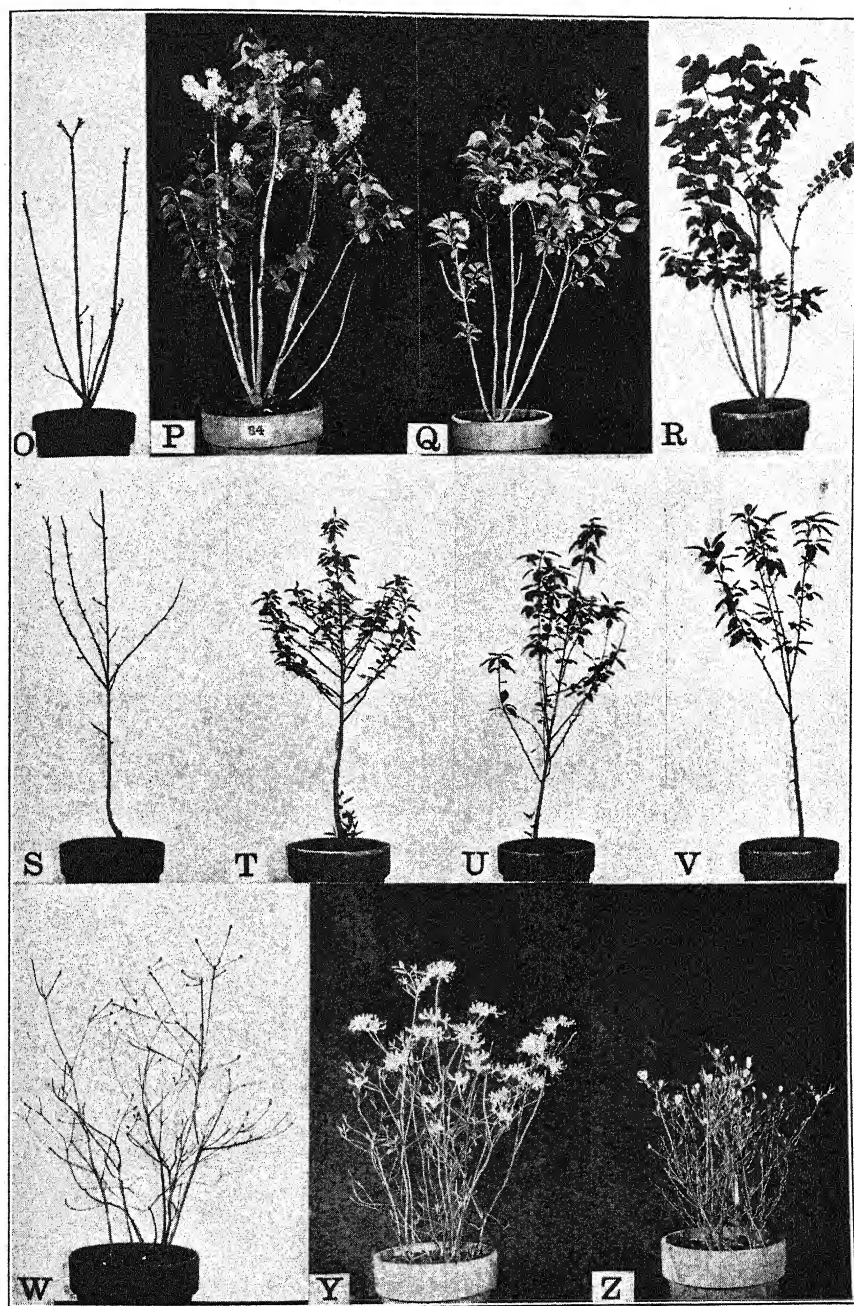
Plant *W* was the check plant, not treated; photographed January 17. Plant *Y* was exposed 24 hours to vapors of ethylene chlorhydrin, 6.7 cc. of the 40-percent solution per 100 liters of space; treated December 23, photographed January 17. Plant *Z* was exposed 24 hours to vapors of ethylene chlorhydrin 0.75 cc. of the 40-percent solution per 100 liters of space; treated December 23, photographed January 17.



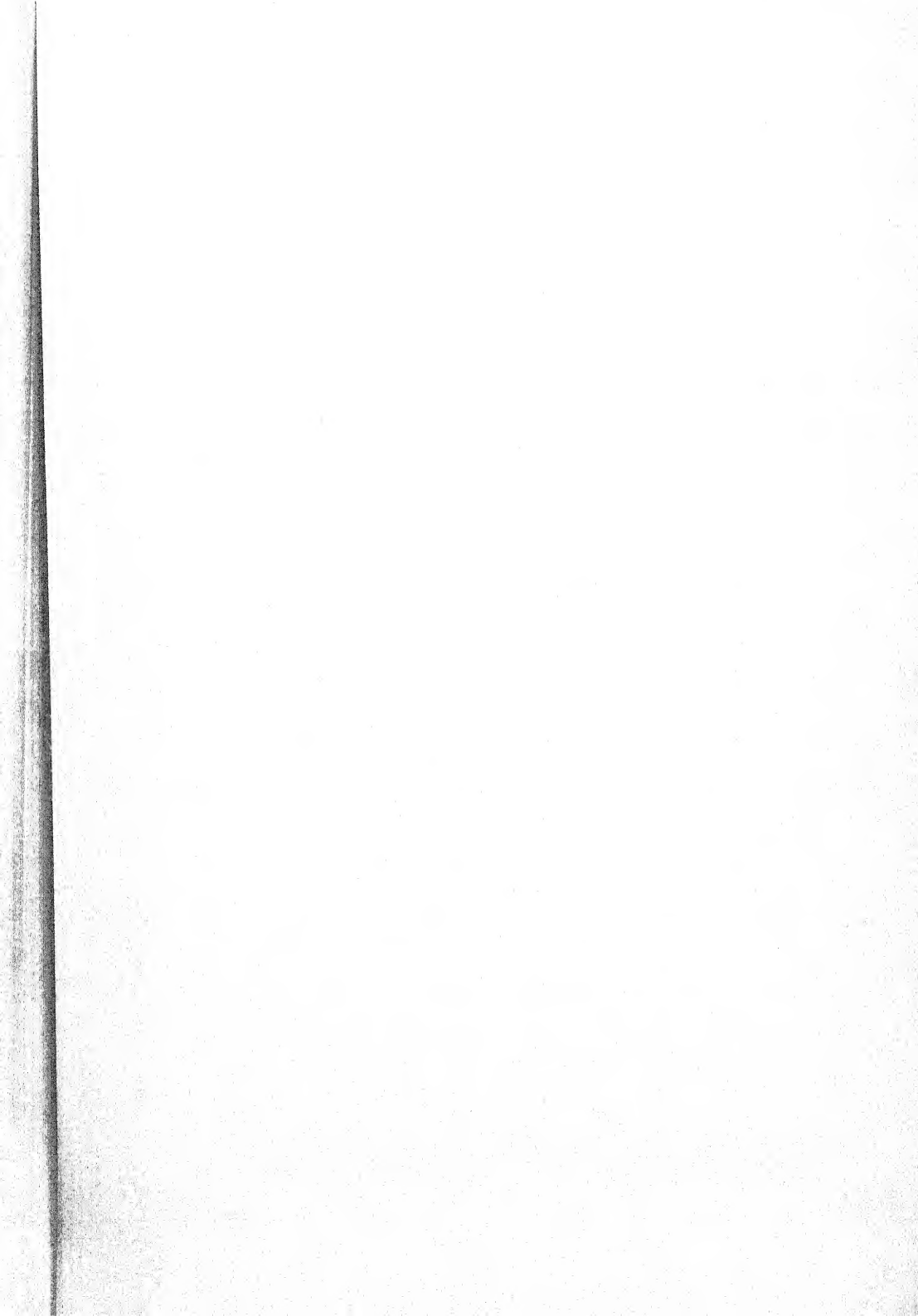
DENNY AND STANTON: REST PERIOD







DENNY AND STANTON: REST PERIOD



## LOCALIZATION OF RESPONSE OF WOODY TISSUES TO CHEMICAL TREATMENTS THAT BREAK THE REST PERIOD

F. E. DENNY AND ERNEST N. STANTON

(Received for publication February 18, 1928)<sup>1</sup>

In the preceding paper (2) it was shown that dormant woody plants when grown in pots and exposed to the vapors of certain chemicals are forced into growth. In the present paper it is emphasized that individual twigs and buds upon the intact plant act independently, and that a single twig or a single bud will respond to this treatment if the selected twig or bud is allowed to come in contact with the vapor, while other twigs or buds are protected from exposure to it.

Lilac (*Syringa vulgaris* L.) proved to be a favorable plant for these experiments since the buds are opposite, and, at the tips of the twigs, are side by side in pairs. It was found possible to treat any one bud of such a pair and to induce the prompt growth of the treated bud without breaking the dormancy of the untreated bud located only a few millimeters away.

The buds or twigs that were started into growth in this way grew vigorously thereafter, showing that the roots and conductive tissues in the stem were not dormant, but were able to supply sap to the buds as soon as the buds were able to use it. The dormancy was not, therefore, systemic; only the buds themselves were dormant.

This view of the matter was first proposed by Howard (3, p. 24) because of the fact that in his experiments twigs without roots responded in a manner similar to rooted plants in pots, and because of the further observation that the buds were always the first organs to show a renewal of activity. By means of the experimental technique described below we obtained evidence that this view is correct.

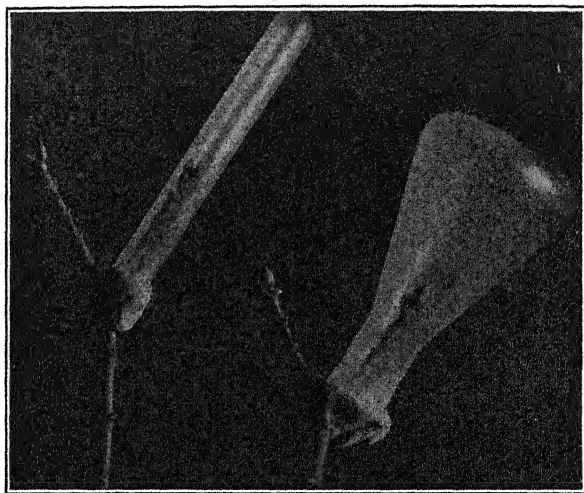
This paper emphasizes how narrowly the effect of the treatment may be localized in the tissue, and that, in fact, the influence of the vapor is upon the buds only.

### TREATMENT OF INDIVIDUAL TWIGS ON THE SAME PLANT

In the preliminary experiments the tips of certain branches upon a plant were exposed to vapors of chemicals by the method shown in text figure 1. Into glass flasks and test tubes of sizes varying from 300 cc. to 28 cc. a drop of chemical was placed; the flask was then inverted over the twig and the opening between the twig base and the mouth of the tube

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

or flask was sealed with molding clay (plasteline). The check consisted in treating other twigs in exactly the same manner except that a drop of water was used instead of a drop of chemical.



TEXT FIG. 1. The method used in exposing individual twigs to vapors. A drop or two of the chemical was placed in the flask which was then inverted over the twig, the seal at the bottom of the tube or flask being made with molding clay (plasteline).

A typical case giving the results of such an experiment is shown in text figure 2. The treatments were made December 10, 1927, and the photograph was taken January 6, 1928. Twig *A* was exposed to vapors of ethylene chlorhydrin for 40 hours by putting one drop of 40-percent ethylene chlorhydrin in a 28-cc. test tube and inverting over the twig in the manner shown in text figure 1. The treatment for twig *B* was one drop of ethylene dichlorid; for twig *C*, one drop of ethyl iodid; for twig *D* one drop of acetaldehyde. For the check, twig *E*, one drop of water was placed in a 28-cc. test tube and the tube was inverted and sealed in a manner similar to that of the treated lots. The other buds upon the same plant may also be regarded as checks, since they received no chemical treatment. It will be seen that only the treated twigs started growth, and it will be noted that the growth was vigorous and healthy. The untreated twigs remained dormant. The activity that was induced in one twig by chemical treatment has not been communicated to neighboring twigs.

Another illustration of this effect is shown in text figure 3. Twig *A* was treated with vapors of ethylene chlorhydrin, using one drop of the 40-percent commercial solution in a 300-cc. flask, inverting the flask over the twig as shown in text figure 1, and letting stand 40 hours. Twig *B* received one drop of ethylene dichlorid in a 300-cc. flask, and twig *D* one drop of ethylene dichlorid in a 50-cc. flask. Twig *C* was the check, receiving



one drop of water in a 300-cc. flask. The treatment was given December 10, 1927, and the photograph was taken January 6, 1928. The interesting comparisons in text figure 3 are twig *A* with twig *E*, twig *B* with twig *F*, and twig *D* with twig *G*. Only the treated twigs started growth. Adjacent and comparable untreated twigs have remained dormant. Position upon the plant has not been an important factor, as is shown by twig *C* in text figure 2 and twig *D* in text figure 3. The chemical treatment has been the deciding factor for the growth of the buds on the twigs.

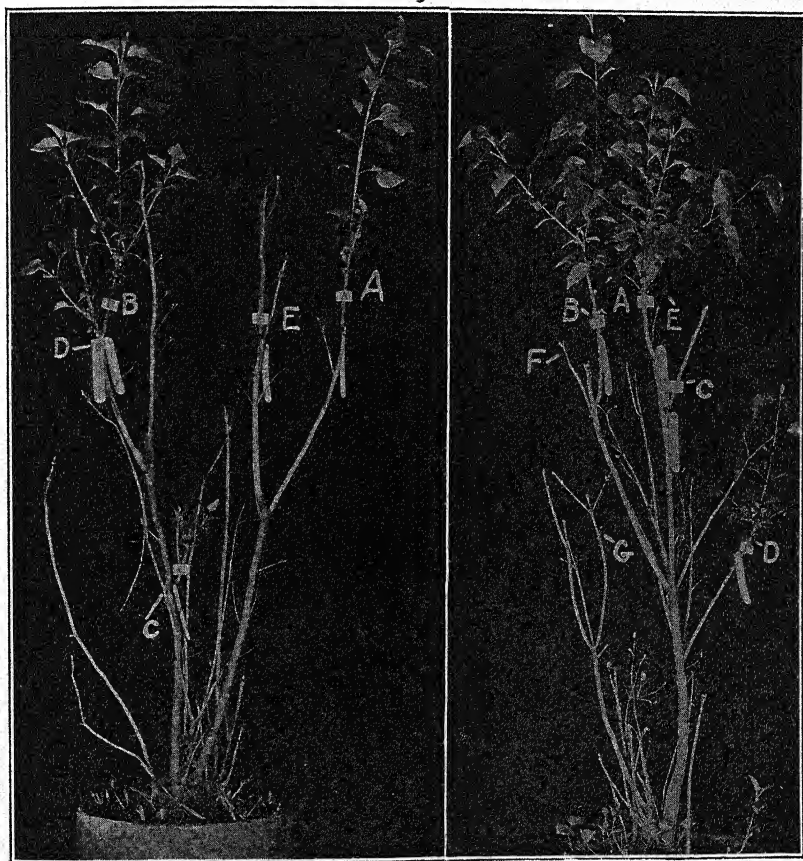


FIG. 2.

FIG. 3.

TEXT FIG. 2. Twig *A* was treated with ethylene chlorhydrin, twig *B* with ethylene dichlorid, twig *C* with ethyl iodid, twig *D* with acetaldehyde, and twig *E* with water. Other buds on the plant may be regarded as checks also, since they received no chemical treatment. TEXT FIG. 3. Twig *A* was treated with ethylene chlorhydrin, twigs *B* and *D* with ethylene dichlorid, and twig *C* with water. Other twigs on the plant, including twigs *E*, *F*, and *G*, were not treated. Compare twigs *A* and *E*, twigs *B* and *F*, and twigs *D* and *G*.

Text figures 2 and 3 show that the twigs upon the dormant plants act as individuals, and that any one twig may be aroused from its dormancy

while other twigs upon the same plant remain in the resting period. Thus, lilac responded to short exposures to vapors in much the same way as blueberry twigs to long periods of low temperatures as described by Coville (1).

#### TREATMENT OF INDIVIDUAL BUDS UPON THE SAME TWIG

The method of treating individual buds upon the same twig is shown in text figures 4 and 5. A disk of molding-clay (plasteline) is first placed



FIG. 4.

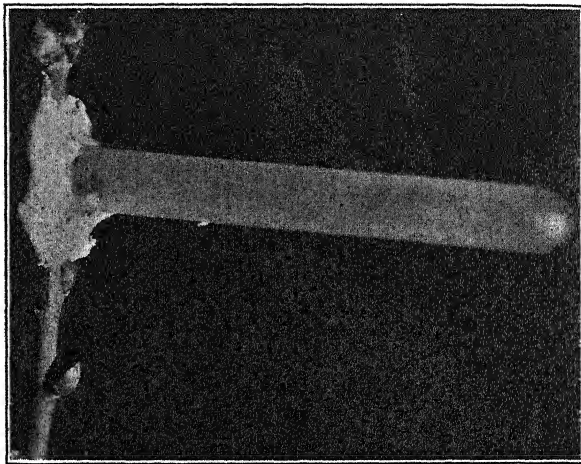


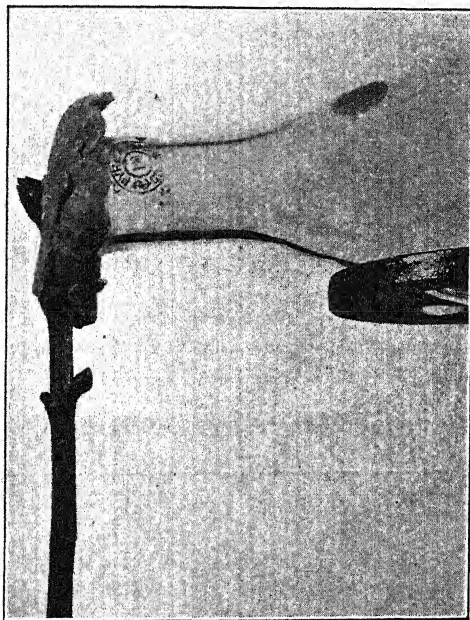
FIG. 5.

TEXT FIG. 4. Method used in treating individual buds. Molding clay (plasteline) was first placed around the selected bud in order that the chemical vapors could be applied to it without having them come in contact with the opposite bud on the other side of the twig.

TEXT FIG. 5. Method of applying chemical vapors to individual buds. A drop or two of chemical was placed in the tube, the mouth of which was then placed over the selected bud and the seal made with molding clay. The bud on the opposite side of the twig was not exposed to chemical vapors.

around the bud, as in text figure 4. The chemical is then dropped into the test tube or flask, and the mouth of the tube or flask is placed against the clay and the edges sealed. Only the bud within the tube is thus exposed to the vapors. The procedure for tip buds is shown in text figure 6. For checks upon this treatment the method is the same except that water instead of a chemical is used, and of course other buds upon the same twig or upon other twigs on the same plant may rightfully be regarded as checks, since they have not been exposed to chemical vapors. Separate experiments in which buds were partly or wholly encased in molding clay (plasteline) showed that contact of the buds with this material is not an important factor in these experiments.

The results of tests with individual buds are shown in text figure 7. Single buds upon twigs of a potted plant of common lilac were treated with ethylene chlorhydrin vapors by the method described in the preceding paragraph. The chlorhydrin solution used was the 40-percent solution diluted 1 : 2 with water; one drop of this solution per 28-cc. test tube was used, and the time of exposure of the buds to the vapors was 24 hours. The treatments were made December 30, 1927, and the photographs were taken January 20, 1928. Only the treated buds started growth. The



TEXT FIG. 6. Method of treating only one bud of the pair of buds at the tip of a twig of lilac. The molding clay layer protects the bud at the left from contact with the chemical vapor in the flask; the right-hand bud of the pair, however, is in contact with the vapors.

opposite bud, in each case located only a few millimeters away, received no treatment and remained dormant. None of the adjacent buds started into growth, showing that the effect of the vapors was narrowly localized in the bud.

The results of an experiment in which buds of the "Charles X" variety of lilac were used are shown in text figure 8. A single bud on each twig was treated with vapors of ethylene chlorhydrin, using the method shown in text figure 6. The ethylene chlorhydrin solution used was the commercial 40-percent solution diluted 1 : 4. In each case two drops of this solution were dropped into the tube or flask used for applying the vapor over the bud. In this series the size of flasks used varied from 10 cc. to 300 cc.





TEXT FIG. 7. Results of applying vapors to only one bud on each twig, using the method shown in text figures 4 and 5. The treated bud on each twig (common lilac) started into growth, but the other buds on the twigs received no chemical treatment and remained dormant. The bark, roots, and wood of the stem were not dormant, but could supply sap to the buds as soon as they could begin growth. Only the buds were dormant.



TEXT FIG. 8. Result of treating only the right-hand bud of the pair of buds at the tip of twigs of "Charles X" lilac, using the method shown in text figure 6. In the case of twig number 8 (counting from the left) the tip buds were not treated, but only the right-hand bud of the pair at the node below the tip. In each case the treated bud was brought out in advance of the untreated bud. For a discussion of such cases as buds number 2 and 7 (counting from the left) see text.

The treatments were applied to buds upon the intact plant; after the buds had made considerable growth the twigs were clipped from the plant and arranged for the photograph (shown in text figure 8) by placing the treated bud at the right and the untreated bud at the left in each case. The localization of the response in the individual bud is again shown in text figure 8. This also shows that the effect upon individual buds can be produced at will, and is not a rare occurrence that can be obtained only occasionally.

In some cases the opposite bud of the pair was also forced into growth. Buds 2 and 7 (counting from the left) in text figure 8 show this effect to a certain extent. Occasionally adjacent buds above and below the treated bud developed in advance of other buds on the same twig or plant. In such cases it cannot be stated whether the clay seal had channels in it that allowed the opposite bud to receive some vapor, or whether the factor or factors that induced the starting of growth in the treated bud were translocated in the stem across to the opposite bud. More experiments with reference to this feature of the work are desirable.

#### DISCUSSION

These results, showing that the effects of the vapor treatments in breaking dormancy are narrowly localized in the buds alone, are helpful to experimenters who are dealing with the causes of dormancy and with the effect of agents that break it. The experiments indicate that the evidence of changes accompanying the resumption of growth in resting plants should be sought in the buds and not in the adjacent tissue; that is, in taking tissue for examination, the bark, cambium, wood, and pith may, for the present at least, be regarded as of secondary importance. For example, in analyzing tissue to determine changes in enzym activity or chemical constituents, it would be undesirable to include in the sample entire twigs or tubers. It would appear likely that the inclusion of the extraneous tissue would obscure any changes that had taken place in the tissue of critical importance, the buds.

Moreover, it appears likely that only certain tissues *inside the buds* are important factors in the changes that are set up by the vapor treatments. Much work needs to be done in localizing these internal effects. Are there changes in the storage tissue within the bud that can be correlated with the resumption of cell division in the growing point? Or do the first detectible signs of change take place in the growing point itself?

#### SUMMARY

1. Individual twigs of dormant lilac plants growing in pots were exposed to vapors of ethylene chlorhydrin, ethylene dichlorid, and ethyl iodid, while similar twigs upon the same plant received no chemical treatment. The treated twigs started growth but the untreated twigs remained dormant.

The separate twigs on a given plant acted as units, and any one of them could be aroused from the rest period while adjacent twigs were inactive.

2. Likewise, individual buds upon twigs were exposed to vapors of ethylene chlorhydrin under suitable conditions, and were forced into growth by this treatment, while the bud upon the opposite side of the twig received no treatment and remained dormant. Of the two buds at the tips of twigs of lilac any one could be caused to develop new twigs without breaking the dormancy of the other bud of the pair.

3. Dormancy in the lilac is not systemic, that is, distributed throughout the plant. It is localized in the buds only. The roots, bark, and conductive tissues are not dormant, but are able to supply sap to the buds as soon as the buds are able to use it.

4. The experiments indicate that, in studying the dormant period, evidence of changes accompanying the resumption of growth should be sought first of all in the buds themselves, and not in the surrounding storage tissue.

5. It is pointed out that more work is needed in localizing these responses more closely *within the bud*.

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## THE IMPORTANCE OF TEMPERATURE IN THE USE OF CHEMICALS FOR HASTENING THE SPROUTING OF DORMANT POTATO TUBERS

F. E. DENNY

(Received for publication April 7, 1928)<sup>1</sup>

### INTRODUCTION

In a previous paper<sup>2</sup> it was shown that early germination of dormant tubers of potato (*Solanum tuberosum* L.) could be obtained by dipping the cut tubers into a dilute solution of ethylene chlorhydrin, and storing the dipped potatoes in a closed container for 24 hours.

Subsequent experiments have shown that the temperature which prevails during the 24-hour storage period after dipping is an important factor. When the temperature was as high as 35° C. (95° F.) severe injury or death of the seed-pieces resulted, and when the temperature was as low as 15° C. (59° F.) the effectiveness of the chemical treatment was considerably lessened. Within the range from 20° C. (68° F.) to 32° C. (90° F.), however, favorable responses were obtained.

Another method of hastening the sprouting of dormant tubers reported upon in the previous paper<sup>2</sup> consisted in soaking the cut tubers in a dilute (1-2 percent) solution of sodium thiocyanate (NaSCN). It has been found that the temperature of the solution used for soaking the tubers was of less importance with this method. Good results were obtained at temperatures from 15° to 30° C.; some unfavorable influence was observed at 35° but serious rotting of tubers did not result even at this temperature.

The object of the present paper is to show the responses given by dormant potato tubers to chemical treatments at different temperatures.

### VARIETY AND SOURCE OF TUBERS

Tubers of the variety Bliss Triumph were used in these experiments. Freshly harvested tubers, dug early in January, 1928, from the fall crop planted in October, 1927, were donated by the Everglades Experiment Station of the University of Florida and were shipped by express to Yonkers.

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

<sup>2</sup> Denny, F. E. Second report on the use of chemicals for hastening the sprouting of dormant potato tubers. Amer. Jour. Bot. 13: 386-396. 1926. See also Contrib. Boyce Thompson Inst. 1: 169-179. 1926.

The potatoes were harvested from green vines and, although of fair size (75 to 150 grams), they were immature and therefore very dormant. The degree of dormancy is indicated by the fact that untreated tubers planted January 10 to January 30 showed only a few sprouts on March 20 (text figs. 3, 7, 8).

#### METHOD OF TREATMENT WITH ETHYLENE CHLORHYDRIN

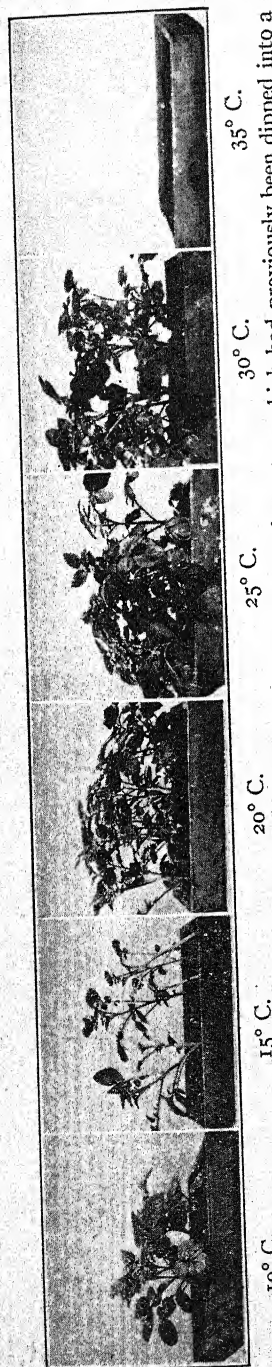
The tubers were first cut into pieces ready for planting. The concentrations of the solutions of ethylene chlorhydrin ( $\text{C}_2\text{H}_4\text{Cl}_2\text{OH}$ ) used for dipping the cut tubers were varied in order to note the effect of different concentrations at different temperatures. The dipping solutions were prepared by adding 60, 30, 15, and 7.5 cc. of 40-percent ethylene chlorhydrin, respectively, to 940, 970, 985, and 992.5 cc. of water. Fifteen seed-pieces were used in each lot and these were placed in glass Mason jars with a large opening ("EZ-Seal" type). The chlorhydrin solution was then poured in until the jar was full and then poured off at once; the jar was inverted and (after placing the hand over the opening) was shaken to remove the excess liquid; the rubber gasket was applied, and the jar was sealed. The jars were then placed in a constant-temperature oven or water bath and let stand for 24 hours. The treated tubers were planted in shallow flats in soil (15 seed-pieces in each flat), were stored in the dark until sprouting began, and were then placed in a greenhouse maintained at a temperature of 18° C. (65° F.).

#### RESULTS WITH ETHYLENE CHLORHYDRIN TREATMENTS

In the first experiment the treatments were carried out at temperatures of 10°, 15°, 20°, 25°, 30°, and 35° C. The treatments were applied January 19-20, sprouts appeared above ground February 19-27, and photographs were taken March 20. These results are shown in text figures 1, 2, and 3, and table 1. The photographs show the size attained by the plants in the different lots, but do not show clearly the percentage germination. Information on this point can be obtained from table 1. The lots treated with a dipping solution consisting of 30 cc. of 40-percent ethylene chlorhydrin per liter are shown in text figure 1; those with a dipping solution of 15 cc. per liter are shown in text figure 2; the check lots dipped in water and stored in the same sort of containers for the same length of time and under the same conditions are shown in text figure 3. Other treatments were carried out with dipping solutions consisting of 60 cc. and 7.5 cc. per liter; part of these results are illustrated in text figure 4 and further information regarding them can be found in table 1.

It is important to note the break in the series which occurs between 30° and 35° C. (text fig. 1). A favorable response was obtained at 30° but at 35° the seed-pieces all rotted. At temperatures below 20° C. the treatments using 30 cc. per liter were less successful; however, when the concen-

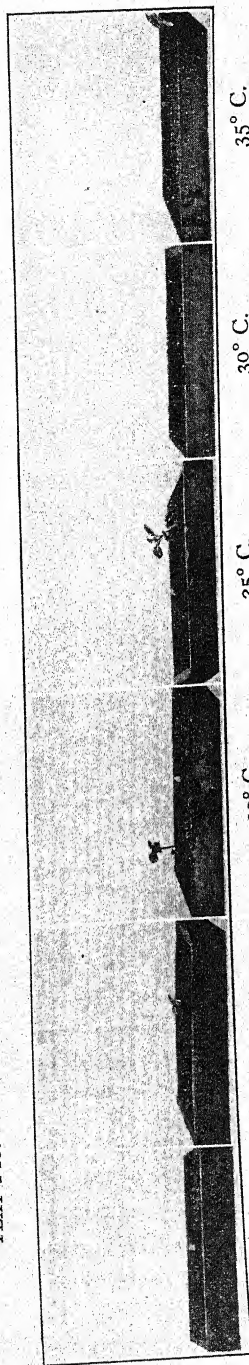




10° C. 15° C. 20° C. 25° C. 35° C.  
 TEXT FIG. 1. Effect of the temperature during the 24-hour period of storage of potatoes which had previously been dipped into a solution of ethylene chlorhydrin containing 30 cc. per liter. Note the break in the series between 30° C. and 35° C. Compare with the check lots at the corresponding temperatures in text figure 3.



10° C. 15° C. 20° C. 25° C. 30° C. 35° C.  
 TEXT FIG. 2. Same treatment as in text figure 1 except that the dipping solution contained 15 cc. per liter.



10° C. 15° C. 20° C. 25° C. 30° C. 35° C.  
 TEXT FIG. 3. Check lots, dipped into water instead of the solution of ethylene chlorhydrin but in all other respects treated in the same way as those shown in text figures 1 and 2. Bliss Triumph variety.

tration of the dipping solution was increased to 60 cc. per liter the germination at 10° C. was improved as is shown by text figure 4 and table 1. The effect of reducing the concentration of the dipping solution to 15 cc. per liter is shown in text figure 2 and table 1. While this series shows much better germinations than the check lots at the same temperatures, the results were not as favorable as those obtained with 30 cc. per liter. Reducing the concentration did not avoid injury at 35° C., and the germinations at the lower temperatures were poor.

TABLE 1. *Influence of Temperature in the Ethylene Chlorhydrin Method of Treating Dormant Potato Tubers*

Temperatures 10° C. to 35° C.					Temperatures 30° C. to 35° C.				
Temp.	Dipping Solution, cc. per liter	Germination Record			Temp.	Dipping Solution, cc. per liter	Germination Record		
		No. Germ.	No. Dormant	No. Rotten			No. Germ.	No. Dormant	No. Rotten
10° C...	60	10	4	1	30° C.	30	15	0	0
" ..	30	5	10	0	" "	15	10	5	0
" ..	15	2	13	0	" "	Water	4	11	0
" ..	Water	0	15	0	31° C.	30	12	2	1
15° C...	30*	7	7	1	" "	15	11	4	0
" ..	15	7	6	2	" "	Water	1	14	0
" ..	Water	1	14	0	32° C.	30	12	1	2
20° C...	60	12	2	1	" "	15	12	2	1
" ..	30	13	2	0	" "	Water	2	13	0
" ..	15	6	8	1	33° C.	30	9	0	6
" ..	Water	1	14	0	" "	15	11	4	0
25° C...	30	12	2	1	" "	Water	2	13	0
" ..	15	9	6	0	34° C.	30	4	0	11
" ..	7.5	10	5	0	" "	15	10	3	2
" ..	Water	1	14	0	" "	Water	1	14	0
30° C...	30	11	3	1	35° C.	30	0	0	15
" ..	15	7	4	4	" "	15	4	2	9
" ..	7.5	7	8	0	" "	Water	2	12	1
" ..	Water	1	12	2					
35° C...	30	0	0	15					
" ..	15	5	1	9					
" ..	7.5	6	4	5					
" ..	Water	0	8	7					

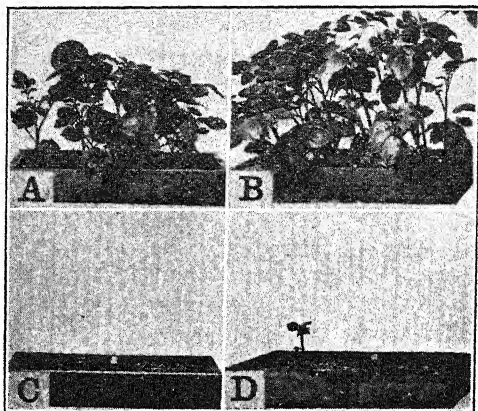
Cut tubers were dipped into a dilute solution of ethylene chlorhydrin and stored 24 hours in a closed container. There were 15 seed-pieces in each lot.

\* The 60 cc. per liter lot at 15° C. was destroyed by accident.

Since it was found in the first experiment that the results at 30° contrasted very sharply with those at 35°, a second experiment was carried out in which this range of temperature was investigated at one-degree intervals. This result is shown in text figures 5, 6, and 7, and table 1.

It is seen that with a dipping solution of 30 cc. per liter, the critical temperature is about 32° C. (90° F.), the results being favorable at 30°, 31°, and 32°, and unfavorable at 33°, 34°, and 35°. Reducing the concen-

tration of the dipping solution to 15 cc. per liter (text fig. 6) permitted fair results at temperatures of 33° and 34°, but there was little indication that any considerable gain was obtainable by making treatments with concentrations below 30 cc. per liter.



TEXT FIG. 4. Lots A and B treated with ethylene chlorhydrin, dipping solution 60 cc. per liter; Lot A at 10° C. (40° F.) and lot B at 20° C. (68° F.). Lots C and D checks, dipped in water instead of ethylene chlorhydrin, but in other respects treated the same as lots A and B; Lot C at 10° C. and lot D at 20° C.

These experiments with the ethylene chlorhydrin show (with the Bliss Triumph variety, at least) that attention must be given to the temperature which prevails at the time of treatment. The temperature of the place of storage for the treated tubers during the 24-hour period after dipping must be below about 32° C. (90° F.) in order to avoid injury to the potatoes, and should be higher than about 15° C. (59° F.) in order to get maximum percentage germination. The disadvantage of low storage temperature can be partly compensated for by increasing the concentration of the dipping solution to 60 cc. per liter, but injury at temperatures above 32° C. cannot be successfully avoided by decreasing the concentration of the dipping solution.

#### RESULTS WITH SODIUM THIOCYANATE TREATMENTS

Battery jars of 3.5 liter capacity were nearly filled with the sodium thiocyanate solutions and the temperature of the liquid was properly adjusted before the experimental tubers (previously cut into pieces ready for planting) were added. The temperature was maintained at the desired degree for one hour by constant attention, and the solution was frequently stirred. The treated tubers were then removed and without being rinsed were planted in the soil in flats. After sprouts appeared above ground, the flats were transferred to a greenhouse maintained at about 24° C.

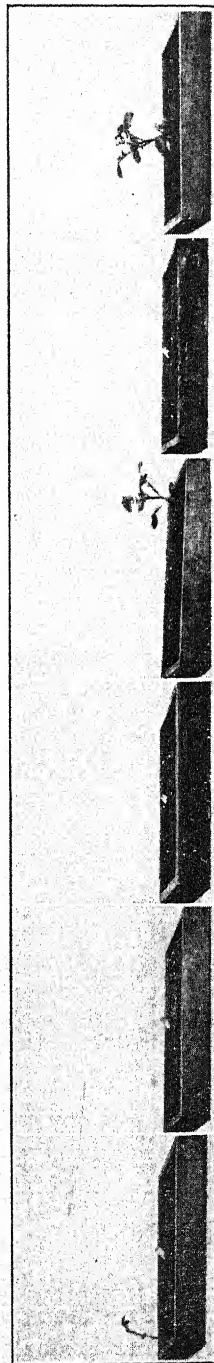




30° C. 31° C. 32° C. 33° C. 35° C.  
 TEXT FIG. 5. Comparison of tubers within the range 30° C. to 35° C. Ethylene chlorhydrin dip method used, dipping solution 30 cc. per liter; dipped tubers were stored in closed containers 24 hours at the temperatures given above. Note unfavorable results at temperatures above 32° C. (90° F.). Compare with the check lots in text figure 7.

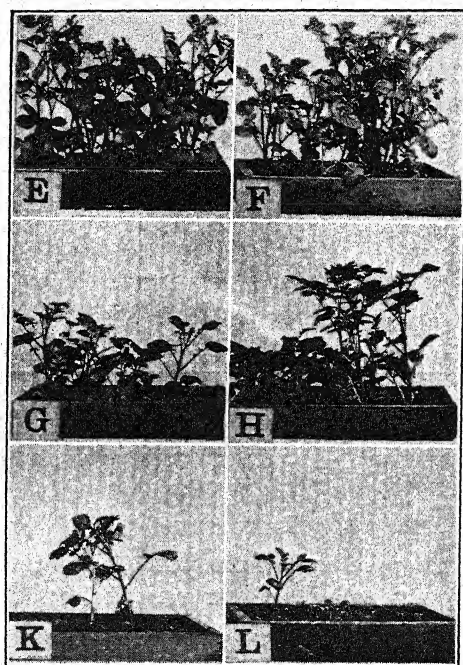


30° C. 31° C. 32° C. 33° C. 35° C.  
 TEXT FIG. 6. Same treatment as in text figure 5, except that the dipping solution contained 15 cc. per liter.



30° C. 31° C. 32° C. 33° C. 35° C.  
 TEXT FIG. 7. Check lots, dipped into water instead of chlorhydrin solution but in all other respects treated in the same way as those shown in text figures 5 and 6. Bliss Triumph variety.

(75° F.). The treatments were made January 27 and the photographs were taken March 20. The results are shown in text figure 8 and in table 2. Only the results at 15° C. and at 30° C. are shown in the figure, but the results at 22° C. were similar in all respects to those at 15° C. and 30° C. In the case of the lots treated at 35° C., it was noted that the tubers soaked in 0.5-percent sodium thiocyanate germinated first, those treated with 1-percent second, and those with 2-percent next. Thus while no serious difficulty with rot was obtained at 35° C. there were indications of some toxicity at this temperature with the stronger concentrations of chemical.



TEXT FIG. 8. Results of treatments with sodium thiocyanate at different temperatures. Lots *E* and *F* soaked 1 hour in two-percent NaSCN, lot *E* at 15° C. and lot *F* at 30° C.; lots *G* and *H* soaked in 0.5 percent NaSCN, lot *G* at 15° C. and lot *H* at 30° C.; lots *K* and *L*, check lots soaked one hour in water, lot *K* at 15° C. and lot *L* at 30° C.

Because of lack of a sufficient quantity of experimental tubers the effect of temperatures below 15° and between 30° and 35° could not be tested in connection with the sodium thiocyanate treatments. The experiments, however, indicated little danger of encountering unfavorable temperatures for these treatments under ordinary conditions.

Attention is specially directed to the favorable germination percentages obtained with the sodium thiocyanate treatments as shown in table 2. It is believed that the layer of sodium thiocyanate which remains on the

tuber after soaking, when rinsing is omitted, acts as a disinfectant and retards the growth of organisms that produce rotting of the tuber. It is proposed to test this point by further experiments.

TABLE 2. *Influence of Temperature in the Sodium Thiocyanate Method of Treating Dormant Potato Tubers*

Temperature	Soaking Solution, g. per liter	Germination Record		
		No. Germinated	No. Dormant	No. Rotten
15° C.....	20	15	0	0
" .....	10	15	0	0
" .....	5	14	1	0
" .....	Water	2	12	1
22° C.....	20	15	0	0
" .....	10	15	0	0
" .....	5	15	0	0
" .....	Water	2	12	1
30° C.....	20	15	0	0
" .....	10	15	0	0
" .....	5	15	0	0
" .....	Water	3	10	2
35° C.....	20	8 *	1	1
" .....	10	8	1	1
" .....	5	10	0	0
" .....	Water	3	7	0

Cut tubers were soaked one hour in a dilute solution of sodium thiocyanate and planted at once without rinsing. There were 15 seed-pieces in each lot.

\* Because of insufficient supply of experimental tubers only 10 pieces in each lot were available for the treatments at 35° C.

## DISCUSSION

These experiments were carried out with Bliss Triumph tubers in the early stages of the rest period. Other varieties, and this variety in the later stages of the rest period, may be found to respond somewhat differently.

If the injuries to the tuber in the chlorhydrin treatment at temperatures above 32° C. (90° F.) are due either to lack of oxygen or to the accumulation within the closed space of carbon dioxid formed by the high rate of respiration induced under such conditions, it may be found possible to obviate the difficulty by storing the dipped tubers under canvas instead of in closed containers. This would permit better aeration during the period of storage. In such a case it may be necessary to increase the concentration of chemical.

In this experiment sodium thiocyanate gave better results than ethylene chlorhydrin. In the hundreds of experiments that have been carried out with these two chemicals the results are sometimes better with one and sometimes with the other. In order to determine the factors which cause this difference in behavior, it is planned to carry out a series of experiments in the summer of 1928.

Although emphasis in this paper has been placed upon the conditions

under which unfavorable results can be obtained, it is evident from an examination of the figures and the tables that the previous reports regarding the capacity of these two chemicals to hasten the sprouting of dormant tubers have been confirmed. The temperatures and concentrations at which treatments were carried out were varied over a wide range in order to find the conditions that were unfavorable, yet almost all of the treated lots were better than the checks. The 16 check lots showed in all an average germination of 11 percent. The 41 treated lots, including all concentrations and all temperatures, gave 66 percent germination; and if only the treatments under favorable conditions are considered, *i.e.* ethylene chlorhydrin with a dipping solution of 30 cc. per liter at temperatures of 20° C. to 32° C., and sodium thiocyanate using a one-percent solution at temperatures of 15° C. to 30° C., the germination percentages are: for ethylene chlorhydrin, 83 percent, and for sodium thiocyanate, 100 percent.

#### SUMMARY

1. This is a continuation of experimental work on the use of ethylene chlorhydrin and sodium thiocyanate for shortening the rest period of potatoes. The object of this experiment was to determine with greater accuracy the influence of the temperature during the process of treating the tubers.

2. When the germination of dormant potato tubers was hastened by dipping the cut tubers into a dilute solution of ethylene chlorhydrin ( $\text{ClCH}_2\text{CH}_2\text{OH}$ ) and storing the dipped tubers in closed containers for 24 hours, the temperature which prevailed during the 24-hour storage period was found to be an important factor.

3. Using a dipping solution obtained by adding 30 cc. of 40-percent ethylene chlorhydrin to 970 cc. of water, favorable results were obtained at 20°, 25°, and 30°, but at 35° C. (95° F.) the seed-pieces were killed by the treatment and rotted subsequently. The range from 30° C. to 35° C. was investigated at one-degree intervals. Good results were obtained at 30°, 31°, and 32° C. (90° F.); but at 33°, 34°, and 35° C., low percentage germinations due to rotting of tubers resulted.

4. At temperatures below 20° (68° F.) a dipping solution of 30 cc. per liter was only partly effective and many seed-pieces remained dormant; better germination percentage was obtained when the concentration was increased to 60 cc. per liter.

5. Experiments were also carried out on the effect of temperature in the sodium thiocyanate method of treating dormant tubers. This consists in soaking the cut tubers for one hour in a dilute solution of sodium thiocyanate ( $\text{NaSCN}$ ). It was found that the temperature of the solution used for soaking the tubers was of less importance with this method. Good results were obtained at 15° C. (59° F.), 22° C., and 30° C. (86° F.) and with all three concentrations of chemical used in the experiment: two-

percent, one-percent, and one-half-percent sodium thiocyanate. At 35° C. (95° F.) some evidence of toxicity was observed at the highest concentration, but no serious difficulty with rotting of tubers resulted.

6. These experiments were carried out with tubers of the Bliss Triumph variety. The experimental tubers were harvested from green vines and were in a deeply dormant condition.

7. Previous experiments showing that treatments with these chemicals hastened the sprouting of dormant potato tubers were confirmed. Untreated tubers showed 11-percent germination 7-8 weeks after planting. The average of all chemical treatments at all concentrations and all temperatures was 66 percent, and the average for the chemical treatments under favorable conditions was 83 percent for the ethylene chlorhydrin treatments, and 100 percent for the sodium thiocyanate treatments.



## RHIZOCTONIA DISEASE ON CERTAIN AQUATIC PLANTS\*

W. S. BOURN AND BERNICE JENKINS

(PLATES XI-XIV AND SIX FIGURES)

### Introduction

The inland waters of Back Bay, Virginia, and Currituck Sound, North Carolina (fig. 1) have long been known to represent one of the most important winter feeding grounds for migratory wild fowl in the United States. Here aquatic duck-food plants, such as *Potamogeton pectinatus* L., *P. perfoliatus* L., *Ruppia maritima* L., *Vallisneria spiralis* L., and *Najas flexilis* (Willd.) Rostk. & Schmidt formerly thrived in great abundance. In 1918, almost simultaneously with the opening and enlargement of the Albemarle and Chesapeake Canal, however, these plants began to die out, and by the end of 1926 vast areas were practically denuded of their aquatic vegetation. This destruction of the plant life has wrought an enormous economic loss upon thousands of the native population, who derived their living from gunning and fishing. Shooting clubs and sportsmen have practically deserted their large investments in the region, since wild ducks and geese in any appreciable numbers are no longer attracted there. At the request of WILLIAM E. COREY, a prominent sportsman of New York, who has been interested in these waters, the Boyce Thompson Institute for Plant Research in 1925 undertook a study of the causes for the disappearance of the aquatic duck-food plants, and early the following year the writers were assigned to the investigation.

The region involved represents an area of approximately 300 square miles of inland waters. At 26 widely scattered stations in this area, periodic analyses have been made to determine the salinity of the water. Field observations have been made almost daily from March to October. Plant specimens have been collected for study

\* Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

at various stages of growth and decay. Experiments to determine the influence of soil, water, and salinity upon the growth and the disease of aquatic plants native to the region have been carried on for more than two years in the greenhouse. Inoculation experiments,

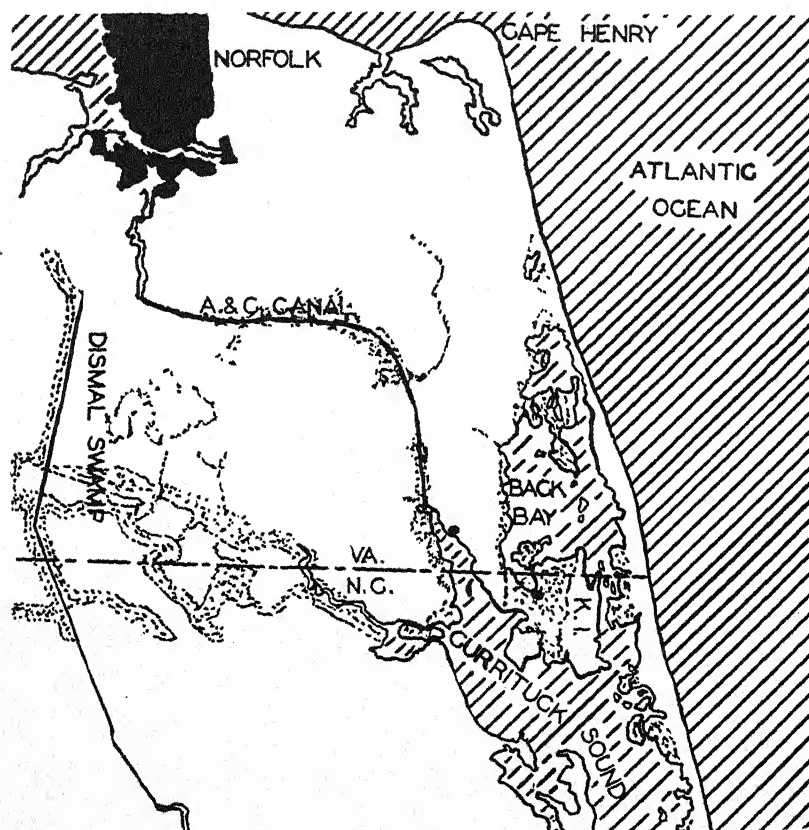


FIG. 1.—Map of Back Bay, Virginia, and Currituck Sound, North Carolina, regions.

using cultures of a fungus isolated from diseased aquatic plants and from diseased potato plants, have extended over the same period of time.

While other injurious factors have been encountered during the course of the investigations, it is the purpose of this paper to describe and discuss a *Rhizoctonia* disease which we believe to be the

factor of greatest importance, and the one most directly responsible for the disappearance of the aquatic duck-food plants in Back Bay and Currituck Sound.

The fungus *Rhizoctonia solani* Kühn is widely distributed in America and elsewhere, having been reported from most regions of the world where the potato is a staple crop. In addition to the potato, which appears to be the most common host, the host plants of this fungus represent many families of dicotyledons and a number of monocotyledons. PELTIER (4) lists about 165 species of plants susceptible to *R. solani*. PALO (3) has recently reported this fungus causing a serious disease of rice in the Philippines. Regardless of the multitude of host plants reported in the literature, which we shall not attempt to review, the occurrence of *Rhizoctonia* on rice plants, to our knowledge, is the closest approach to an occurrence on aquatic plants thus far reported. When we consider such a wide range of hosts, and the fact of its occurrence on semi-aquatic plants, the discovery of *R. solani* in a purely aquatic situation is less surprising, if unusual.

L. O. KUNKEL, pathologist of the Boyce Thompson Institute for Plant Research, first isolated pure cultures of *Rhizoctonia solani* from diseased aquatic plants collected from Back Bay, Virginia, in September 1925. Since that time we have repeatedly isolated pure cultures of this fungus from diseased plants collected from the infected area. We have inoculated plants growing in the greenhouse aquaria with sclerotia from these cultures. These inoculated plants became infected almost immediately, and later pure cultures of the fungus were re-isolated from them. We have determined that this aquatic strain of *Rhizoctonia* will not only kill aquatic plants but will also attack such terrestrial plants as the potato; furthermore, that a strain of *R. solani*, isolated from diseased potato plants, will attack aquatic plants.

#### Occurrence and identification of *Rhizoctonia* on diseased and dead tissue

In the field it was noticed that signs of *Rhizoctonia* infection first became visible as dark lesions on portions of the plant stems, near the first node at the surface of the soil. Infection took place on this



portion of the plant regardless of the depth of the water in which the plant was growing. By taking one-fourth inch segments of these diseased stems, washing them half a dozen times in several cubic centimeters of sterile distilled water, to remove water bacteria, etc., and then transferring these segments to potato and to green string bean agar, pure cultures of *R. solani* were obtained. After the segments had been in the agar one to two days, the fungus could be observed growing out into the agar from the cut ends of the segments. At the end of the fourth day the growth was about the size of a half-dollar, and very shortly thereafter the entire plate became covered by the mycelial growth. About the second week small white spots, 1-2 mm. in diameter, appeared in the older mycelia near the center of the agar plates. Within a few more days these white spots became the black sclerotia shown in pl. XI. Concentric zonations are characteristic of these cultures, and showed plainly in most of them. In measurements of hyphal and sclerotial cells, in mycelial and cultural characteristics, and in physical appearances this fungus corresponds closely to the descriptions of *R. solani* given in the literature. The aquatic strain and the potato strain of *Rhizoctonia* are compared in the cultures shown in pl. XI. *A* and *B* are cultures of the fungus isolated in 1926 from diseased aquatic plants collected from Back Bay, on potato agar and on green string bean agar respectively; *C* and *D* are cultures on potato and green string bean agar respectively of the aquatic strain of *Rhizoctonia* re-isolated in 1927 from greenhouse aquatic plants used in the inoculation experiments; *E* and *F* represent cultures of *R. solani* isolated from diseased potato plants, on potato and on green string bean agar respectively.

**Inoculations with *Rhizoctonia*; production of the disease;  
subsequent re-isolation of the fungus**

To determine the effects of *Rhizoctonia* inoculations upon five species of aquatic duck-food plants, *Potamogeton pectinatus*, *P. perfoliatus*, *Ruppia maritima*, *Vallisneria spiralis*, and *Najas flexilis*, experiments were set up in the greenhouse aquaria. In these experiments four types of soil were used. Water solutions were made up by diluting boiled Long Island Sound water with distilled water

until the solutions contained a saline content equal to the average for Back Bay, to one-half, and to double that concentration. Ten gallons of water was used in each culture. This furnished two feet of water over six inches of soil in the vessels. The form of the plants used in these cultures was either seeds, tubers, or young plants. Inoculations were made by placing in the soil sclerotia of the strain of *Rhizoctonia* isolated from Back Bay aquatic duck-food plants. The results obtained for the inoculation cultures, using *Potamogeton pectinatus*, are given in table I.

TABLE I  
RESULT OF INOCULATION OF POTAMOGETON PECTINATUS WITH AQUATIC STRAIN  
OF RHIZOCTONIA SOLANI

SOIL	SALINITY OF WATER IN PERCENTAGE OF NORMAL SEA WATER	AVERAGE LENGTH OF PLANT LIFE	
		Inoculated	Check
Sterile quartz sand.....	3.5	45 days	100 days
Sterile quartz sand.....	7.0	20 days	100 days
Sterile quartz sand.....	14.0	35 days	90 days
Sterile Back Bay soil.....	3.5	No growth*	No growth*
Sterile Back Bay soil.....	7.0	No growth*	No growth*
Sterile Back Bay soil.....	14.0	No growth*	No growth*
Untreated Back Bay soil.....	3.5	40 days	200 days
Untreated Back Bay soil.....	7.0	25 days	100 days
Untreated Back Bay soil.....	14.0	30 days	100 days
Sterile garden soil.....	3.5	40 days	2 years
Sterile garden soil.....	7.0	30 days	2 years
Sterile garden soil.....	14.0	30 days	2 years

\* Failure of growth due to iron bacteria which grew so abundantly that growth of other plants was prevented.

For the experiments with the other four species of plants mentioned, no tabular results are given. These experiments were set up later and all the checks are still living. The average length of life of the inoculated plants, however, approximates the averages given for *Potamogeton pectinatus*, with the exception of *Vallisneria spiralis*. The absence of a stem above the ground in this plant aids in the prolongation of life, as it is necessary for the fungus to destroy the dozen or more leaves before the plant dies. This plant lived in the inoculated cultures from two to several weeks longer than the species of *Potamogeton*. From the diseased plants of these experiments pure cultures of *Rhizoctonia solani* were re-isolated.

The preceding experiments were run in duplicate with a strain

of *Rhizoctonia* isolated from diseased potatoes. No appreciable differences were observed, however, between the results of these experiments and those in which the aquatic strain of the fungus was used for the inoculations.

#### Relation of salt content of water to incidence of the disease

In order to determine the influence of different degrees of salinity of the water upon the *Rhizoctonia* disease, tubers of *Potamogeton pectinatus* were cultured in glass museum jars with boiled Long Island Sound water diluted with distilled water to the following concentrations: no sea water, 3.5, 7.0, 10.5, 14.0, 17.5, 20.0, 25.5, 27.0, 30.5, and 40.0 per cent normal sea water. The concentrations were checked by the titration method described by DENNY (1). No soil was used in this experiment. Three tubers were cultured in each jar, the tubers being weighted down by sterile stones (pl. XII). One of the tubers was inoculated by making a slight cut and inserting sclerotia of the fungus in the wound; a second tuber was inoculated merely by tying sclerotia on to it; the third tuber of each culture was not inoculated. For these inoculations the aquatic strain of *Rhizoctonia* was used. Duplicate experiments were run, using for the inoculations pure cultures of *Rhizoctonia* isolated from diseased potato plants.

*Rhizoctonia* isolated from aquatic plants was found to attack the plants in all salt concentrations employed in the experiment. The disease appeared more virulent, however, in those concentrations between 7 and 20 per cent normal sea water. In none of these cultures did plants live longer than six weeks. All three tubers of each culture became equally infected; injuring the tuber apparently did not aid the infection.

Pl. XII shows four plants two weeks after inoculation with the aquatic strain of *Rhizoctonia*. *G* is a plant cultured in fresh water; *H* in 3.5 per cent normal sea water; *I* in 7 per cent sea water (the concentration of Back Bay); and *J* in 14 per cent sea water. Pl. XIII shows plants of the same cultures, in the same order, four weeks after inoculation. Photographs for these plates were taken through the museum jars and the water solutions in which the plants were cultured.

The strain of the fungus isolated from diseased potatoes attacked the plants in all concentrations used in the experiment. The disease induced by this strain, however, was most destructive to the plants cultured in fresh water and in the concentrations below 10.5 per cent sea water. While plants cultured in the higher concentrations were attacked, death did not occur until long after the plants had fruited. In pl. XIV, *O* represents plants of a 10.5 per cent normal sea water culture; *P* those of a 27 per cent normal sea water culture. Both photographs were taken three weeks after inoculation with *Rhizoctonia solani* isolated from diseased potato. The facts that this strain of the fungus attacks aquatic plants growing in salt solutions of lower concentrations and that the aquatic strain attacks those in the higher concentrations indicate that the two are separate physiological strains of *Rhizoctonia*.

#### Effect of aquatic strain of *Rhizoctonia* upon potatoes

In order to make a more complete comparison between the potato and the aquatic strains of *Rhizoctonia*, it now remained to study the effects of the aquatic strain upon potato plants. For this purpose potatoes were grown in sterile soil under bell jars. A part of the seed pieces were inoculated in the eyes, at the time of planting, with sclerotia of the fungus. Other inoculations were made in the stems of plants just beneath the surface of the soil. Within a short time dark brown lesions, or sunken areas, appeared on the stems of the inoculated plants, and many underground stolons were completely severed. Upon the appearance of the stem lesions the bell jars were removed and the plants were left to grow for several weeks, until tubers had formed. Upon examination, sclerotia of the fungus were discovered on these tubers, many of which were badly scabbed and russeted (fig. 2).

The results obtained in all the preceding experiments with the two strains of the fungus check very closely. For this reason, there is a possibility that the aquatic plants in Back Bay and Currituck Sound have become infected with *Rhizoctonia* from diseased potatoes. While a large district in which potatoes are grown on a commercial scale drains into these waters, and *Rhizoctonia* is known to occur in these potato fields, we do not know that this is the source of

infection. There seems to be just as strong a possibility that the source of the fungus may be found in the great amount of sewage that enters these waters from the city of Norfolk by way of the Albemarle and Chesapeake Canal.

#### Seasonal developments of the disease

Evidences of *Rhizoctonia* infection are usually observed each year during the first half of June on the *Potamogeton pectinatus* plants growing in the northern part of Currituck Sound and in Back Bay.

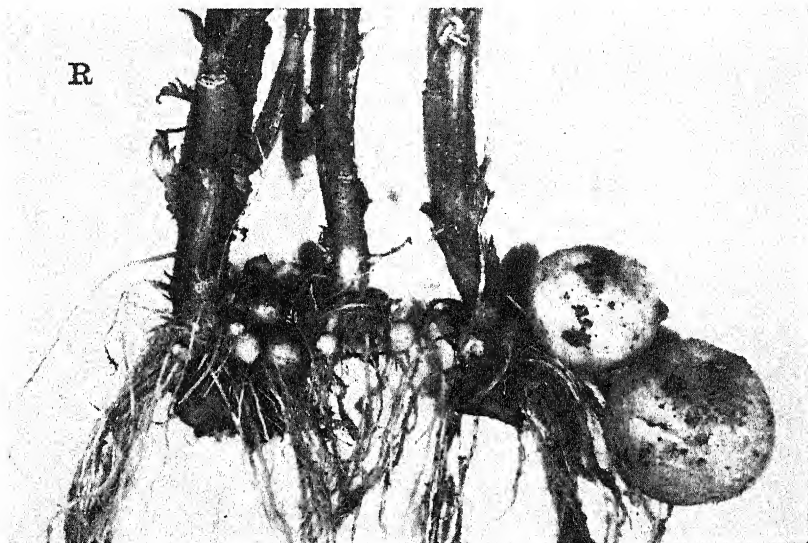


FIG. 2.—Effect of aquatic strain of *Rhizoctonia* upon potatoes

During this month these plants are rather well covered by gelatinous remains of old hydroid colonies which infest the plants a little earlier in the season. At this time of the year the *Rhizoctonia* infections are somewhat limited to dark brown lesions on lower portions of the plant stems, but during the warmer months of August and September the effects of the disease become quite marked in the entire appearance of the plant. We have been able to isolate pure cultures of the fungus from these diseased plants in the months of June, July, August, and September. During the months of August and September the salinity of the water is usually highest (fig. 3). Great

areas of the plants, which up to this time of the season have appeared green, become brown and die within a few days. While the increased salinity of the water may lower the resistance of the plants to the disease, it can hardly be said to kill them, for we have been able to grow the same plants in much saltier water than that found in Back Bay. Furthermore, the dead areas of plants are usually very

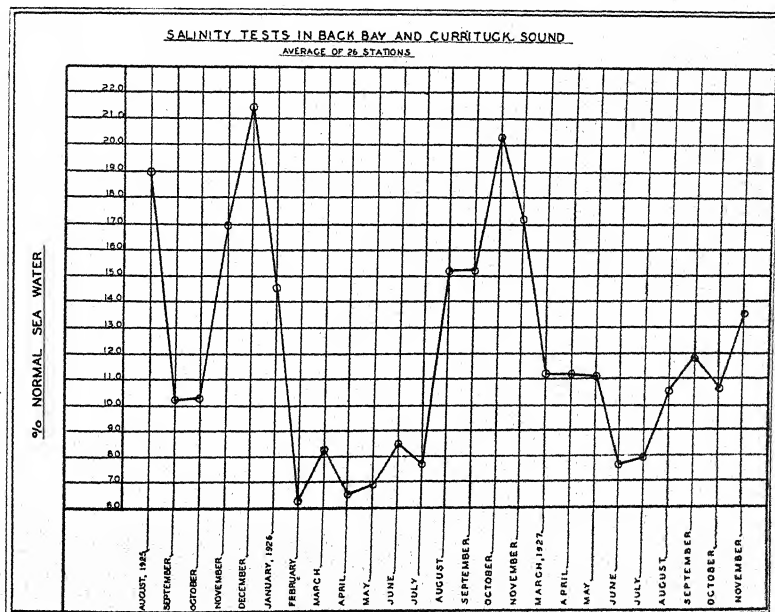


FIG. 3.—Graph showing results of monthly salt analyses of waters of Back Bay and Currituck Sound; readings in percentage of normal sea water, representing averages for 26 widely scattered stations in the region.

irregular, and injuries are restricted to definite portions of the plant itself.

The depth of the water does not effect the development of the disease. *Rhizoctonia* has been found to attack the plants growing in all depths found in Back Bay and Currituck Sound. The depth of these waters, however, does not usually exceed ten feet. In this region there is considerable wind and wave action, and diseased plants, which have lost their anchorage, are easily washed from one area to another. Plants more or less isolated are easily broken off



after infection at the surface of the soil, and are washed ashore while still green; but where the growth is denser the plants remain attached and sink to the bottom after complete decay. In the greenhouse inoculation experiments the plants probably remained attached and alive much longer than they would have done in nature, because wave and wind action was not encountered.

#### Susceptibility of different plants to the disease

While *Rhizoctonia* has been found to attack most of the species of duck-food plants in Back Bay and Currituck Sound, *Potamogeton pectinatus*, which is the dominant plant of the region, and, incidentally, the most valuable duck-food known, is the most susceptible to the disease. This species is the first to make a growth in the spring, and usually reaches the surface of the water, even in the greater depths, before growth begins in other species of duck-food plants mentioned. Isolated areas of *Vallisneria* and *Naias* do not readily become infected with the disease, but infection does occur when these plants are associated with *Potamogeton*. Next to *P. pectinatus*, the plant most susceptible to the disease is *Ruppia maritima*. In this locality the *Ruppia* zone, while in shallower water, usually overlaps the *Potamogeton* zone. Because of its ease in vegetative propagation, however, *Ruppia* usually survives where the *Potamogeton* dies. Plants of all species studied seem less susceptible to the disease when growing, somewhat sheltered from the winds, in sandy soil.

#### Mycelial and sclerotial characteristics of fungus

A microscopic examination, as well as a cultural isolation, of the infected portions of the plants reveals hyphae of *Rhizoctonia*. Under natural conditions mycelial threads are seldom so abundant as to be discernible by the naked eye, but are quite visible in aquaria cultures (pls. XII and XIII). The older external hyphae (fig. 4) are somewhat colored, usually a uniform yellowish brown, with branches arising at approximate right angles to the main hypha; the internal hyphae are usually smaller, practically colorless, and the branching is more or less dichotomous. The younger hyphal branches (fig. 5) are practically colorless and contain large globules. Microchemical tests indicate that these globules contain glycogen. The hyphal

branches are almost invariably constricted, and contain a septum near the point of union with the main hypha (figs. 4, 5). The size of the mycelial cells, taken from 100 measurements, is  $50-225 \times 5.50-12 \mu$ . DUGGAR (2) gives  $100-200 \times 8-12 \mu$  for the size of the hyphal cells of *Rhizoctonia solani*. PELTIER (4) found the size of the hyphal cells of the different strains of the fungus was  $65.24-5.01-6.57 \mu$ ,

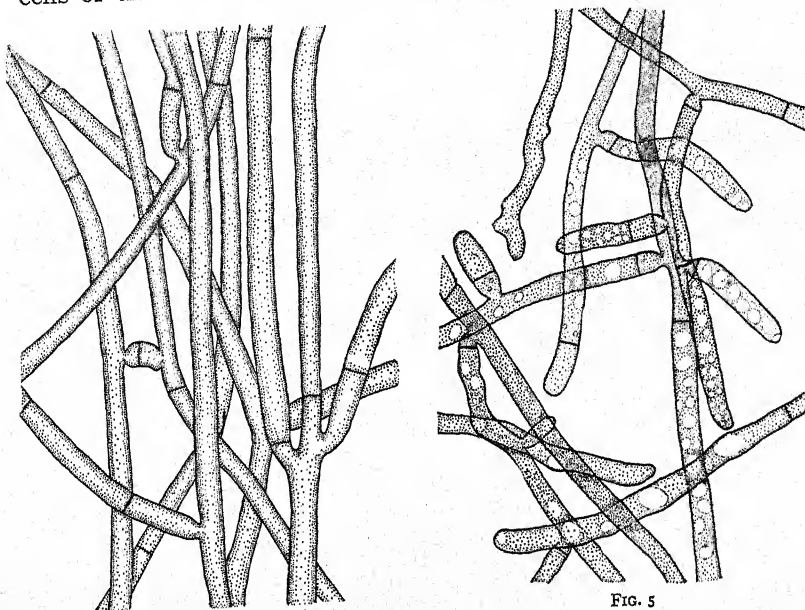


FIG. 4

FIG. 5

FIGS. 4, 5.—Fig. 4, *Rhizoctonia solani*, camera lucida drawing of portion of mycelium growing on aquatic plant; fig. 5, *Rhizoctonia solani*, camera lucida drawing of young hyphal branches of mycelium growing on aquatic plant.

but gave for the length of the cells of the strain parasitic upon *Dianthus*  $50-215 \mu$ . PALO (3) states that the measurements of the hyphal cells of the strain parasitic upon rice are  $24-248 \times 4-12.8 \mu$ . The majority of our measurements for the aquatic strain fall within the range given by DUGGAR.

Hyphae of the infecting mycelia readily penetrate the tissues of the aquatic plants. These penetrating hyphae appear greatly swollen at points of entry or exit from the cells of the host tissue, but a very constricted portion of the hyphae penetrates the cell walls of the host plant.



The exact conditions under which sclerotia may occur on the plants in their native habitat have not been determined. Sclerotia have been observed on the dead plants which float ashore in the late summer. In pl. XIV, *Q* is a photomicrograph of a small stem of an aquatic plant containing sclerotia of the fungus. This plant was fixed in chrom-acetic fixative and stained *in toto* with eosin and

Ehrlich's haematoxylin. Sclerotia first appear on the plants which have washed ashore as light spots bordered by a dark brownish ring of discolored host tissue. These light spots later become dark specks. Some of the sclerotial cells are irregular, flattened, dark, and more or less granular in structure; others, especially of the loose hyphae covering the sclerotia, are barrel-shaped, less colored, and are vacuolate (fig. 6). It is assumed that the sclerotia overwinter in the muck and débris at the bottom of the water. PALO, in reporting the occurrence of *Rhizoctonia solani* upon Philippine rice, states that the viability of the sclerotia is destroyed

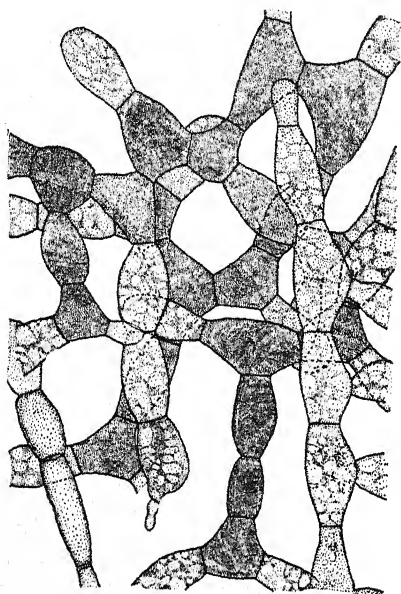


FIG. 6.—*Rhizoctonia solani*; sclerotial cells found on aquatic plant; camera lucida drawing made from stained specimen.

after its submergence in water for a period of two to three months. We have succeeded in obtaining infection without re-inoculation in aquaria two years after the soil in these vessels had been inoculated with sclerotia of the aquatic strain of *Rhizoctonia*. This may be due to a prolonged mycelial growth of the fungus, however, as we were unable to determine that sclerotia had formed in the aquaria.

### Summary

1. Great areas of aquatic duck-food plants have been destroyed in Back Bay, Virginia, and Currituck Sound, North Carolina. Di-

rectly or indirectly, this has caused a large economic loss to a large population. A fungus disease has been found the factor most responsible for this loss.

2. Pure cultures of a strain of *Rhizoctonia* have been repeatedly isolated from diseased aquatic duck-food plants collected from the infected waters. A disease, identical with that produced by a strain of *R. solani*, has been produced in greenhouse aquaria plants by inoculations with this aquatic fungus. Pure cultures of the fungus have again been re-isolated from these greenhouse plants. Moreover, a disease has been produced on potato plants by inoculating the plants with sclerotia from culture of this aquatic fungus.

3. This aquatic strain of *Rhizoctonia* has been found to attack five species of aquatic duck-food plants: *Potamogeton pectinatus*, *P. perfoliatus*, *Ruppia maritima*, *Vallisneria spiralis*, and *Naias flexilis*. Attacks have occurred on plants growing in various types of soils, and in waters containing a wide range of salt content. Muck soils and a salinity of 7-20 per cent normal sea water, however, are most favorable for the growth and activity of the fungus. These suitable conditions of soil and salinity now exist in Back Bay and Currituck Sound.

4. Morphological and cultural characteristics indicate that this fungus is a physiological strain of *Rhizoctonia solani*, and it is so considered by the writers.

5. To our knowledge, this is the first case ever reported of the occurrence of *Rhizoctonia solani* on aquatic plants.

6. A strain of *Rhizoctonia solani* isolated from diseased potatoes has been found to attack aquatic plants growing in various types of soils and salt solutions. This disease was identical with that produced by the aquatic strain of the fungus, but occurred in lower salt concentrations.

7. Under favorable conditions *Rhizoctonia solani* is an aggressive parasite upon those species of aquatic plants studied in this investigation, and the disease induced by it has caused much damage to these plants growing in the infected waters.

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## EXPLANATION OF PLATES XI-XIV

## PLATE XI

Three weeks' old cultures of *Rhizoctonia solani*: *A* and *B*, cultures of fungus isolated in 1926 from diseased aquatic plants collected from Back Bay, on potato agar and on green string bean agar respectively; *C* and *D*, cultures on potato and green string bean agar respectively, of aquatic strain of *Rhizoctonia* re-isolated in 1927 from greenhouse aquatic plants used in inoculation experiments; *E* and *F*, cultures of *R. solani* isolated from diseased potato plants, on potato and on green string bean agar respectively.

## PLATE XII

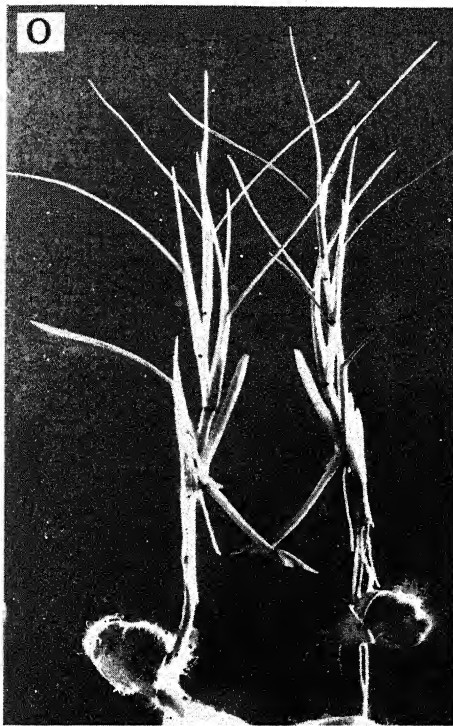
*Potamogeton pectinatus* plants two weeks after inoculation with sclerotia of aquatic strain of *Rhizoctonia*; *G*, plant cultured in fresh water; *H*, in 3.5 per cent normal sea water; *I*, in 7 per cent.

## PLATE XIII

Same as pl. XII four weeks after inoculation; these are plants from the same cultures and arranged in same order as in pl. XII.

## PLATE XIV

*Potamogeton pectinatus* plants (*O*) growing in 10.5 per cent normal sea water three weeks after inoculation with sclerotia of *Rhizoctonia* isolated from diseased potato plants; *P*, same as preceding except plants are growing in 27 per cent sea water; *Q*, photomicrograph of sclerotia of aquatic strain of *Rhizoctonia* on small stem of *P. pectinatus*; plant found washed ashore in Back Bay, Virginia; specimen fixed in chrom-acetic fixative and stained with eosin and Ehrlich's haematoxylin.

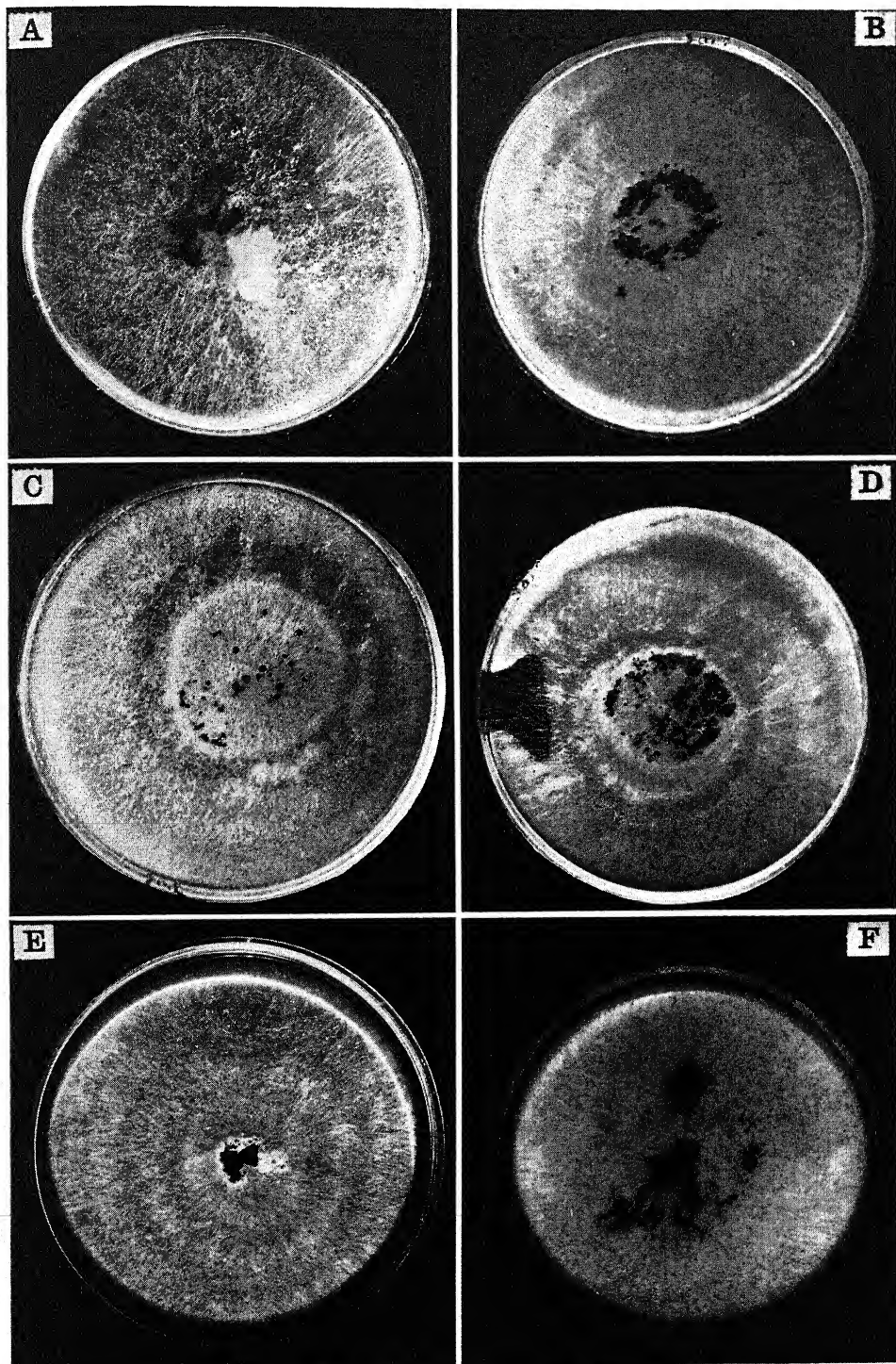


Q



BOURN & JENKINS on RHIZOCTONIA

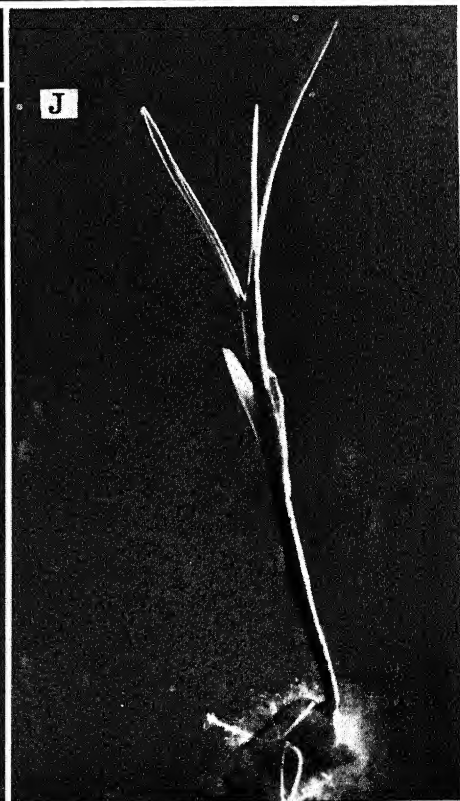
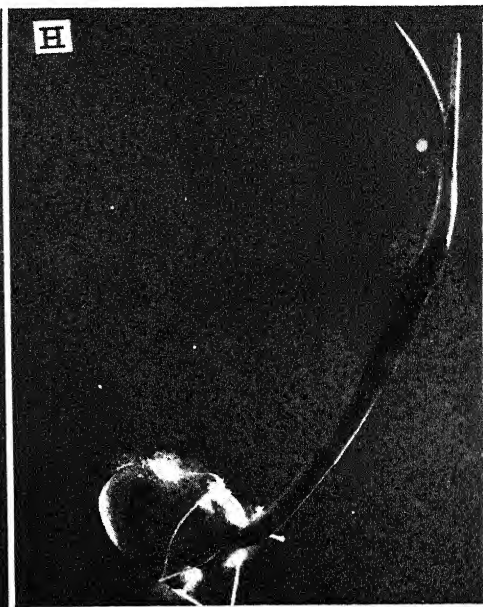
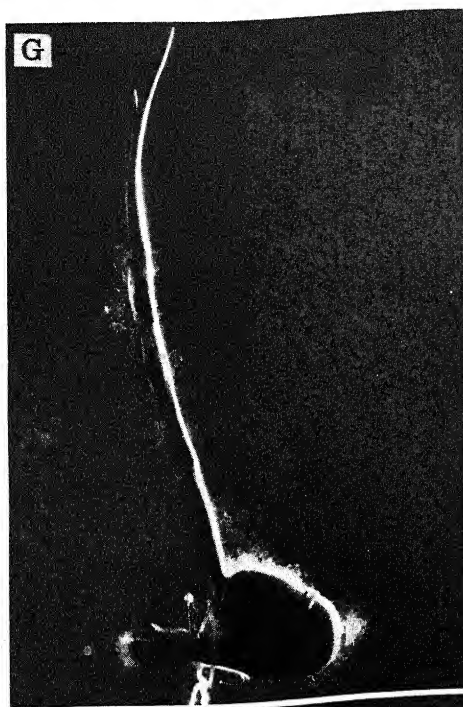




BOURN &amp; JENKINS on RHIZOCTONIA

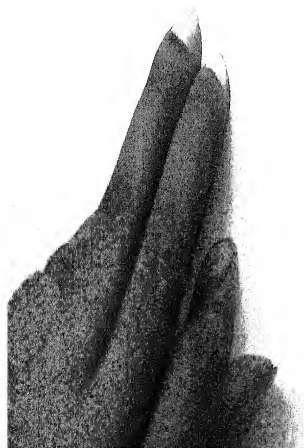


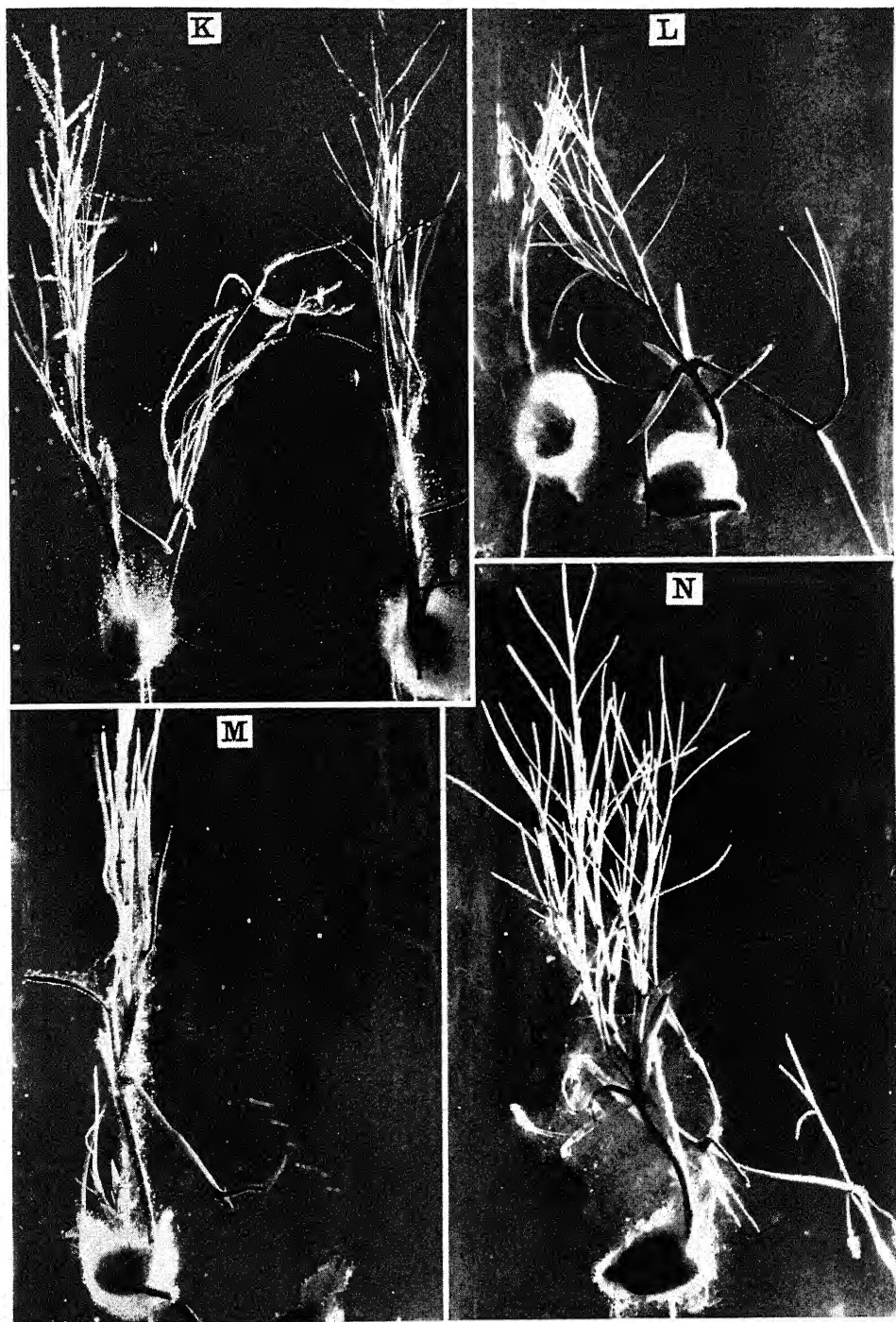




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## ANATOMICAL STUDY OF PLANTS GROWN UNDER GLASSES TRANSMITTING LIGHT OF VARIOUS RANGES OF WAVE LENGTHS<sup>1</sup>

NORMA E. PFEIFFER

(WITH PLATES XV-XVIII AND ONE FIGURE)

A series of plants was grown in glass houses at Boyce Thompson Institute for Plant Research in the summer of 1927, for physiological experiments. A few of the plants were available for anatomical study, the results of which are here recorded. The houses in which the plants were grown have been previously described and figured by POPP.<sup>2</sup> Briefly, each house is 9×11 feet, with roof sloping to the south, with a difference of 2 feet in height at front and back of benches. The ventilation system is of the positive pressure type, electrically driven, with separate air intakes in each house and separate roof ventilator outlets. This results in temperatures which are approximately similar throughout the line of houses, although not identical with that existing out-of-doors in full sunlight.

### Transmission of glasses used in houses

The glasses used in the houses in this experiment transmit light in the visible spectrum and in the ultra-violet in various ranges of wave length. The designations used for these houses, the trade name of the glasses,<sup>3</sup> and the ranges of each within these two spectral regions are as follows:

	Millimicrons
Visible-spectrum house.....Noviol O.....	720-390
Full-spectrum house.....Corex.....	720-290
Blue house.....G403ED.....	585-335
Minus-violet house.....Noviol C.....	720-471
Red house.....G 34.....	720-526

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

<sup>2</sup> POPP, HENRY W., A physiological study of the effect of light of various ranges of wave length on the growth of plants. Amer. Jour. Bot. 13:706-736. 1926.

<sup>3</sup> Corning glasses.

The spectrum of sunlight in the same regions is from 720 to 400 millimicrons in the visible spectrum, and from 400 to 290 millimicrons in the ultra-violet. The light in the full-spectrum house most nearly approaches this in quality of rays, transmitting the ultra-violet rays to the extent of about 80 per cent of their value in full sunlight. The blue house is limited in transmission in the visible spectrum to some yellow, green, and blue, with less rays in the ultra-violet than window glass transmits. The minus-violet house allows the passage of the red, yellow, green, and part of the blue of the visible spectrum. In the red house, only red, yellow, and part of the green of the visible spectrum are transmitted. The last two houses mentioned lack all ultra-violet rays, the visible-spectrum house most of them, and the blue house glass transmits less than window glass. Thus all four fail to transmit the rays valuable to higher animals because of their antirachitic effects. The blue and red house glasses transmit in common only some yellow and some green rays.

In addition to these five glass houses, two other locations served for comparison. One was an adjacent platform, commonly termed "outdoors," where plants received full insolation. Another was a "shade" house, a light frame structure covered with a double layer of gauze. This stood between the spectral glass houses and the "outdoors" plants, also having a southern exposure. These two situations did not have the similarity of temperatures that obtained in the glass houses.

### Intensities

The measurements of intensities are those made by J. M. ARTHUR and H. L. SHIRLEY, using the Macbeth illuminometer and a pyrheliometer. The latter records radiant energy in terms of heat; the former is more specifically a measure for illumination. It is not entirely satisfactory, however, in that the eye, by means of which intensities are gauged, is not ideal in its ability to distinguish degrees of difference equally in various regions of the spectrum. Moreover, the color effect within the glass houses makes accurate readings even more difficult. Neither instrument records light in terms of the plant and physiological effects. Table I represents the averages of readings made at various times, based on the two methods. The light outdoors in each case was considered as 100 per cent intensity,

whether in terms of foot candles (Macbeth illuminometer) or millivolts (pyrheliometer).

### Anatomical studies

From the range of plants available in the different sets of conditions, several were selected for a study of the anatomical effects produced by the light factor. So far as possible, plants had been grown from seed, although when necessary small plants, as 1-inch salvias, represented the stage at the beginning of the experiment. In all cases 2-gallon jars with forest soil were used for the plants. The beginning date of the experiment was June 13, 1927.

TABLE I  
INTENSITIES IN THE VARIOUS SITUATIONS

SITUATION	MACBETH ILLUMINOMETER Per cent	PYRHELIOMETER Per cent
Outdoors.....	100	100
Visible-spectrum house.....	59.8	52.7
Full-spectrum house.....	55.0	50.8
Blue house.....	8.6	8.0
Minus-violet house.....	50.7	33.2
Red house.....	31.3	30.2
Shade house.....	16.2	23.2

STEMS.—When the plants were six weeks old, the stems of a number were examined, especially *Mirabilis jalapa*, *Brassica rapa* (Henderson's Purple Top White Globe), *Helianthus cucumerifolius* (type of Henderson), and *Glycine soja* var. *biloxi*. The first two proved least satisfactory for accurate comparison of degree of development of vascular tissues, because of the scattered arrangement of unequal bundles in four-o'clock, and because of the short stem in turnip which makes it difficult to get comparable preparations. Yet it was possible to see in these the same general effects that were more readily demonstrable in the typically dicotyledonous stems of the sunflower and soy bean. Later collections, made when the plants were approximately fourteen weeks old, again included these two forms.

Free-hand sections were made of the stem at the base and just below the middle node. These show similar trends, as may be seen in the illustrations from photomicrographs, taken at the fourteen-week

stage (pls. XV-XVIII). Study was made of two stems, of which one set was photographed. In comparing results, the full-spectrum house is considered the control, since it has the spectrum of sunlight and the temperature is similar to that of the other four houses. The different temperature outdoors and in the shade house bring in another factor which would eliminate the use of these as checks.

In soy bean (pls. XV and XVI) the stem in the full-spectrum house compares very favorably with that outdoors, and is noticeably stronger in development of woody tissues than in any other house. The plants in the shade house and minus-violet house are much alike, both a little less strong than those of the visible-spectrum house. Undoubtedly the plants in the blue and red houses represent the weakest development. That the least differentiation occurs in the blue house may be due as much to the very low intensity as to the special rays which reach the plant.

In the case of the sunflower (pls. XVII and XVIII) the full-spectrum house plant is not so strong in development of differentiated tissues as either the outdoors plant or that of the visible-spectrum house. There is little difference in intensity in the two houses, but that little is in favor of the visible-spectrum location. Whether this could be of more importance to a "sun" form like *Helianthus* than to other types, it is impossible to say. The plants in the minus-violet house also had well developed xylem, although here intensity is less high. It would have been interesting to compare the shade form in this case, but unfortunately all plants were used before the final anatomical study. The earlier stage showed weaker vascular tissues under these conditions than the minus-violet plants. In all cases the plants in the blue and red houses were least differentiated. In the upper region the former showed no secondary wood, although at a corresponding level the latter had a fair amount. With the low intensity in the blue room, it is very possible that the material for elaborating differentiated tissues is lacking. It is conceivable that the available food may be the limiting factor, and that the quality of light is not directly responsible for such weak development. Certainly the appearance of the plant, a sort of miniature of those in the full-spectrum house, is more nearly normal than that of plants in visible-spectrum, minus-violet, or red houses.



Aside from the actual amount of differentiated tissues produced, the dimensions of the stem prove of some interest. Considering the diameter of the base of the stem of the full-spectrum house as 100 per cent, we get the results shown in table II. Here the sunflower and four-o'clock appear to respond in similar fashion, with the exception of sunflower in the outdoors situation. Compared with these two, the soy bean makes a better showing, so far as diameter is concerned, in both the red house and in shade. This is not necessarily due to development of differentiated tissue, but chiefly to pith.

TABLE II  
DIAMETER OF BASE OF STEMS (SIX WEEKS OLD) AS COMPARED WITH  
FULL-SPECTRUM HOUSE

HOUSE	FOUR-O'CLOCK Per cent	SUNFLOWER Per cent	SOY BEAN Per cent
Outdoors.....	95	150	97
Visible-spectrum.....	82	81	70
Full-spectrum.....	100	100	100
Blue.....	56	50	60
Minus-violet.....	78	75	70
Red.....	43	49	67
Shade.....	66	74	83

It is well to associate height with diameter. In table III the same plants considered in the previous table are listed on this basis, again with the full-spectrum plant as 100 per cent. Here also sunflower and four-o'clock appear more nearly parallel, whereas soy bean reacts quite differently. It endures low intensities better than the other two forms, as shown by diameter and height. On the other hand, where abnormal form is brought about by lack of the blue and ultra-violet rays, soy bean is even more affected (as shown by twining) than the other two genera.

Table IV records a comparison of heights from the more extensive physiological data collected by J. M. ARTHUR. The previous table is based on two samples, while this is an average of a greater number (2 to 7 more) of plants. In table V there is the same general trend, although minor differences appear in the figures.

LEAVES.—In form the leaves in the minus-violet and red houses appear puffed and warped, although they are ordinarily of very good

size. In the blue-house plants, on the other hand, the greatest reduction in leaf expanse runs parallel to the small size of the whole organism. The leaves in the other houses, which are intermediate between these two extremes, fall into this order, on the basis of expanse: second largest in visible and full-spectrum houses, next in outdoors and shade houses.

TABLE III  
HEIGHT OF STEMS (SIX WEEKS OLD) AS COMPARED WITH  
FULL-SPECTRUM HOUSE; SAME STEMS AS TABLE I

HOUSE	FOUR-O'CLOCK Per cent	SUNFLOWER Per cent	SOY BEAN Per cent
Outdoors.....	85	85	57
Visible-spectrum.....	104	100	144
Full-spectrum.....	100	100	100
Blue.....	57	26	91
Minus-violet.....	100	110	181
Red.....	110	49	185
Shade.....	54	66	115

TABLE IV  
AVERAGE HEIGHT OF STEMS (SIX WEEKS OLD) AS COMPARED WITH  
FULL-SPECTRUM HOUSE

HOUSE	FOUR-O'CLOCK Per cent	SUNFLOWER Per cent	SOY BEAN Per cent
Outdoors.....	103	95	60
Visible-spectrum.....	115	98	149
Full-spectrum.....	100	100	100
Blue.....	53	25	82
Minus-violet.....	103	97	141
Red.....	120	46	168
Shade.....	83	65	100

In considering the thickness of leaves, the extremes fall in the outdoors plants, where the leaves are thickest, and in the red house, where they are least thick. In two of the three forms measured (soy bean and four-o'clock), those growing in the full-spectrum house rank highest among the houses, that is, most nearly approaching outdoors plants; while the visible-spectrum plants made a close second. In the case of sunflower, the relation in these two houses is

just reversed. The minus-violet and shade leaves tally with each other very well, and the blue-house leaves, with their small size, prove but little different from these. The order here is (from the thickest to least thick): full-spectrum, visible-spectrum, blue, minus-violet and shade, and red houses. The shade and minus-violet sunflowers are aberrant.

Here it is obvious that quality of light is effective in reducing the thickness of the red-house leaves, since the intensity (table I) is intermediate between that of the blue house and the minus-violet house, both of which have thicker leaves.

TABLE V  
THICKNESS OF LEAF

HOUSE	FOUR-O'CLOCK Per cent	SUNFLOWER Per cent	SOY BEAN Per cent
Outdoors.....	110	154	133
Visible-spectrum.....	84	107	80
Full-spectrum.....	100	100	100
Blue.....	68	79	77
Minus-violet.....	66	100	66
Red.....	48	79	63
Shade.....	66	102	66

In studying the differentiation of the leaves, one finds that in sunflower and soy bean two layers of palisade cells ordinarily develop in outdoors conditions (fig. 1). In this feature, the full-spectrum leaves resemble those outdoors very definitely; while in the visible-spectrum house, lacking the ultra-violet rays, the second layer is weaker in soy bean. In all the other houses there is reduction to one layer, and in shade, with ultra-violet rays available, although in low intensity, we again find a vague second layer produced.

In four-o'clock (fig. 1) the long compact palisade layer, seen in outdoors leaves and in full and visible-spectrum leaves, does not develop in the other houses. In the shade house it is of intermediate length, while the cells in the minus-violet house are even shorter; in the blue house they are very open; and in the red, very short. In all cases leaves in the blue and red houses show a markedly poorer development than those in full or visible-spectrum houses. The weakest differentiation occurs in the red house.

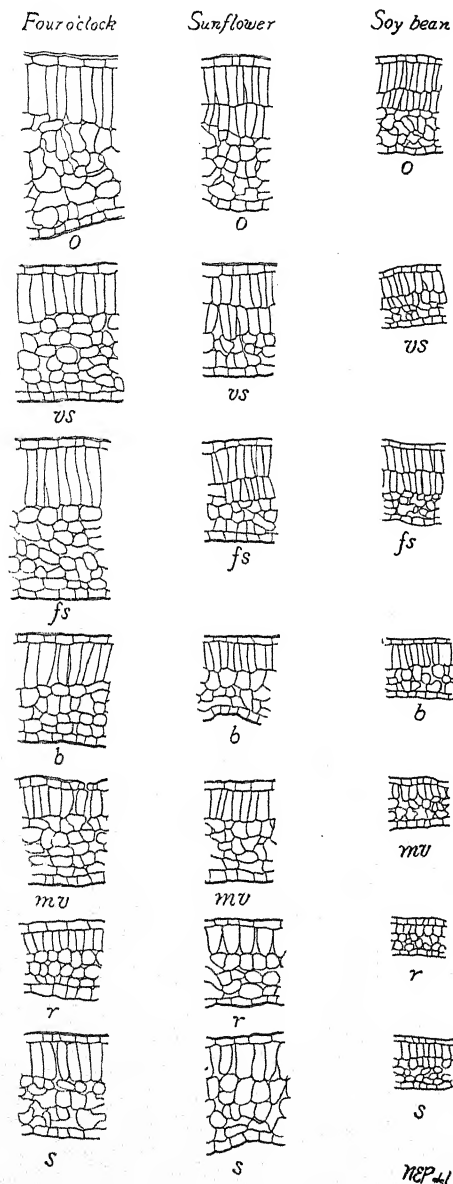


FIG. 1.—Sections of leaves of four-o'clock, sunflower, and soy bean from plants grown in different houses; houses are indicated by the following letters: *o*, outdoors; *vs*, visible-spectrum house; *fs*, full-spectrum house; *b*, blue house; *mv*, minus-violet house; *r*, red house; *s*, shade.

**Roots.**—It seems worth while to consider roots, but so far the material available renders it difficult to make any statement based on measurements, such as were made of the other organs. One may make an estimate on the basis of length, thickness, and development of secondary roots. Using this method, it was found that the outdoors plant takes precedence over any of the glass-house plants. Of the latter, the full-spectrum plant ranks first, the visible-spectrum next, the minus-violet plant (to which shade plants are similar) third, with the plants in the blue and red houses decidedly poor and feeble in comparison.

### Discussion

It is difficult to make valid comparisons of plants in different situations where more than one factor varies. Hence the first two houses, which have approximately equal intensities, are the best ones on which to base conclusions as to the effect of quality of light. It seems evident that the plant in the full-spectrum house makes a better growth, so

far as differentiated tissues (as shown in vascular tissues in stem, diameter of stem, and thickness of leaf) are concerned; and it produced the usual form of plant, with the tissues commonly found in full insolation. The plant in the visible-spectrum house is weaker in vascular development, more spindly as to stem, with greater height, less thick as to leaf, and with poorer root development. The difference in light, that is, lack of most of the ultra-violet rays, results in this less stocky, less sturdy, more watery plant.

The same effect on form is noticeable in the two houses which emphasize the red end of the spectrum. Here, however, different

TABLE VI

SUMMARY: DEGREE OF DEVELOPMENT LISTED IN ORDER WITH THE HIGHEST FIRST

STEM			LEAF		ROOT
Vascular development	Diameter	Height	Thickness	Differentiation	Development
Outdoors.....	Full-spectrum	Visible-spectrum	Outdoors	Outdoors	Outdoors
Full-spectrum...	Outdoors	Full-spectrum	Full-spectrum	Full-spectrum	Full-spectrum
Visible-spectrum	Visible-spectrum	Minus-violet	Visible-spectrum	Visible-spectrum	Visible-spectrum
Minus-violet....	Minus-violet	Outdoors	Blue	Shade	Minus-violet
Shade.....	Shade	Blue	Minus-violet*	Blue	Shade
Blue or red.....	Blue or red	Red and shade vary in position	Shade*	Minus-violet	Blue
Red or blue....	Red or blue		Red	Red	Red

\* Exception in sunflower.

intensities, that is, lower ones, enter in as a factor, and may be partly responsible for the very weak stems, for the poor differentiation and thinness of leaf, and for the slight development of root.

It is unfortunate that in the blue house it has not been possible to obtain an intensity more nearly comparable with that of the full-spectrum house. At present it is impossible to dissociate the effects of quality and intensity of light in this house. In form the plants appear like miniatures of full-spectrum plants. Microscopic examination, however, shows very much weaker differentiation and development of tissue in stem and leaf. Whether the amount of food is the real limiting factor, that is, an indirect effect of light, or whether the light itself directly determines this, is not known.

### Summary

Some sort of diagram, which would bring all these organs and their development into one view, seems a useful sort of summary.

Table VI serves this end. Usually outdoors plants take the first place, with the full-spectrum plants usually second, and visible-spectrum plants third. Except in height, which is the least tractable of the figures to handle, minus-violet-house and shade plants usually fall close together, and the blue- and red-house plants are generally at the end of the series, showing weaker development and differentiation. In the full-spectrum and visible-spectrum houses quality of light determines the difference, since the intensities are similar. In the remaining houses, both quality and intensity may be effective in bringing about the changes.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.  
YONKERS, N.Y.

#### EXPLANATION OF PLATES XV-XVIII

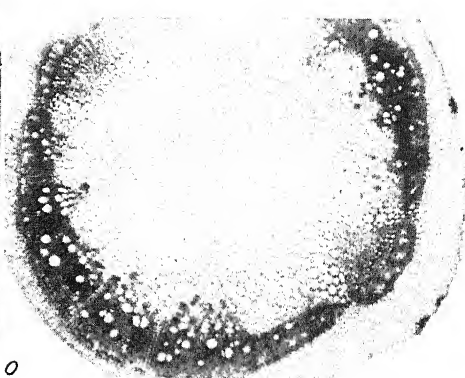
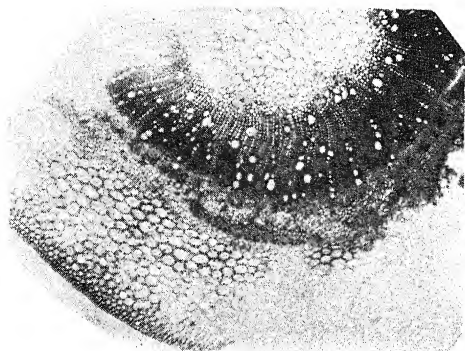
All photomicrographs of free-hand sections of stems were made with the use of the Leitz Makam micro-camera. In all plates the first column, *B*, shows the base of stem; the second, *M*, shows the middle of stem, half-way between base and apex. The smaller letters indicate locations in which the plants were grown: *o*, outdoors; *vs*, visible-spectrum house; *fs*, full-spectrum house; *b*, blue house; *mv*, minus-violet house; *r*, red house; *s*, shade. Plants were 14 weeks old.

##### PLATES XV, XVI

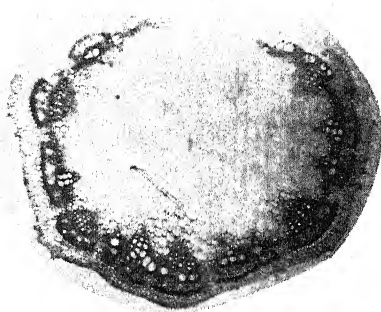
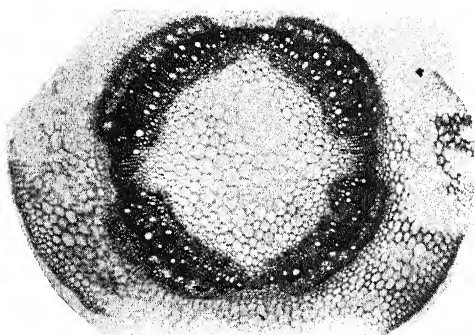
*Glycine soja* var. *biloxi*.

##### PLATES XVII, XVIII

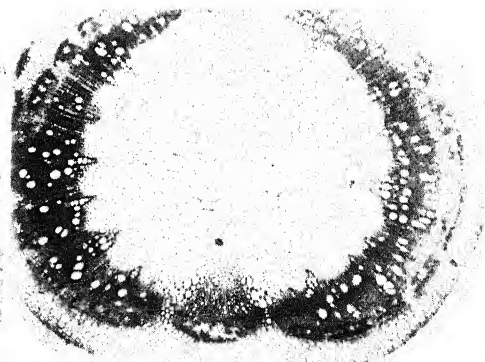
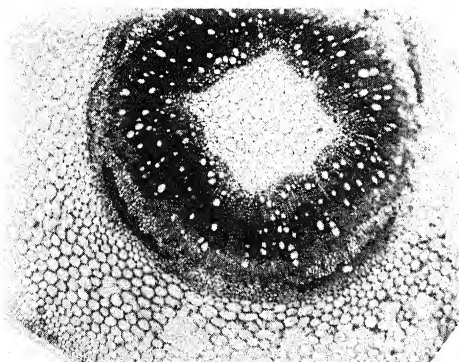
*Helianthus cucumerifolius*, type of Henderson.



*o*



*v s*



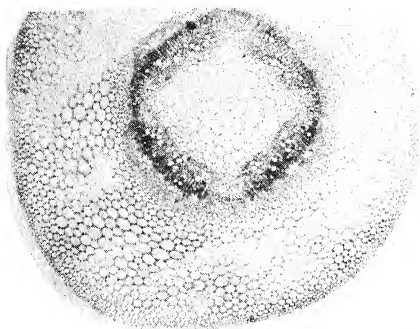
*f s*

*B*

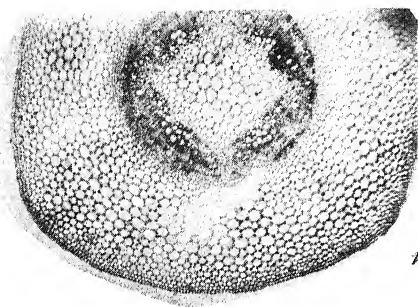
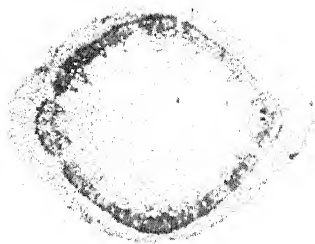
*M*



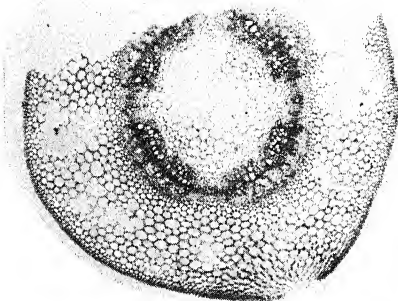
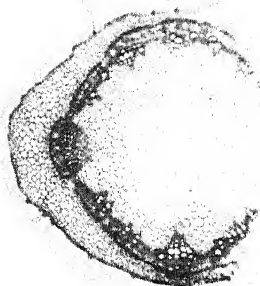




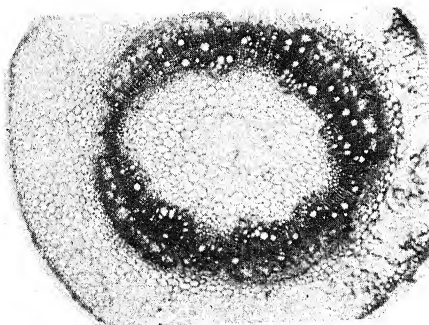
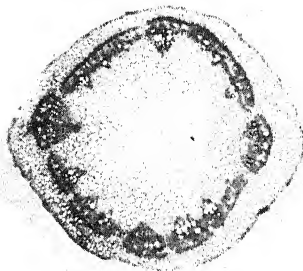
*b*



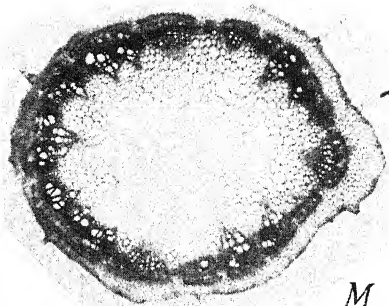
*m v*



*r*

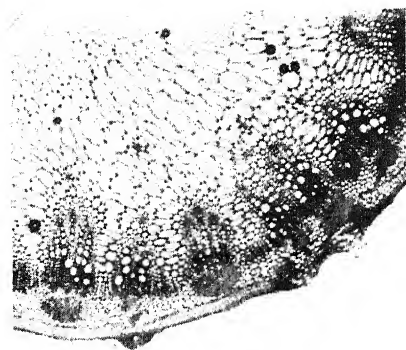
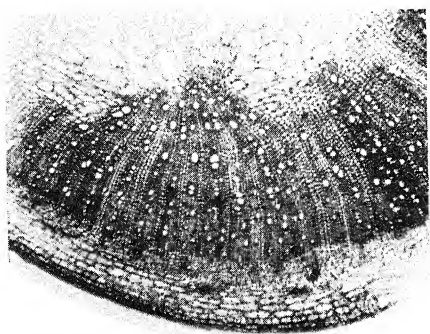


*s*

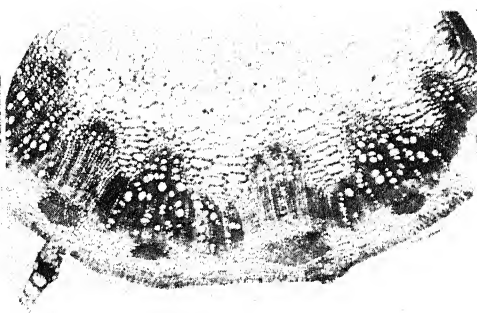
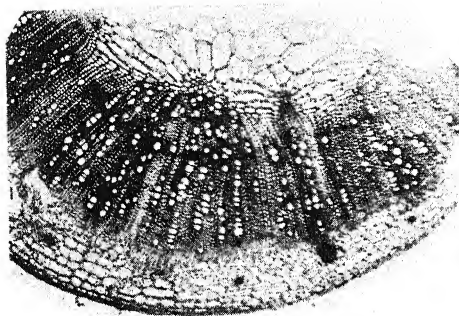


*M*

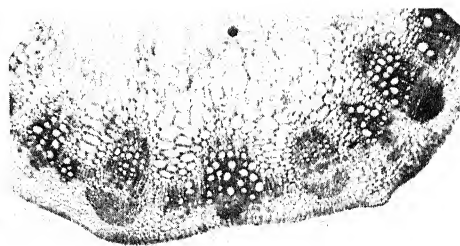
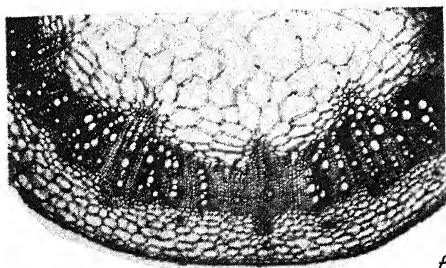




*o*



*v s*



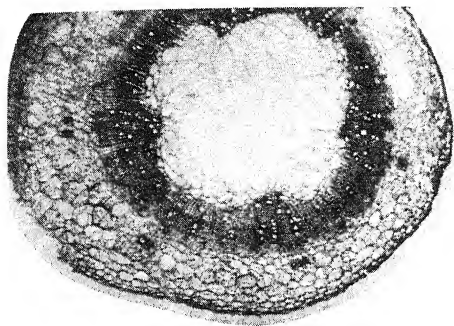
*f s*

*B*

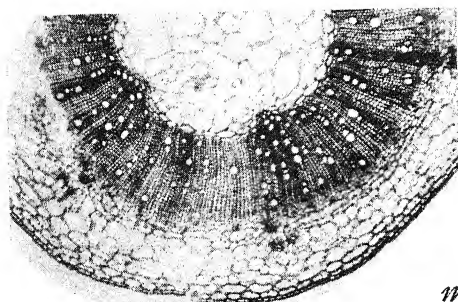
*M*

PFEIFFER on PLANTS AND LIGHT

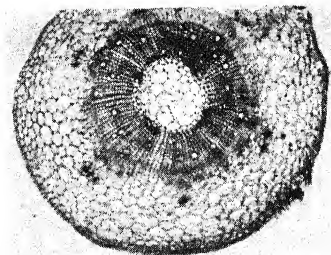
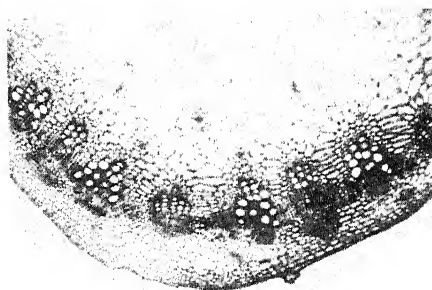




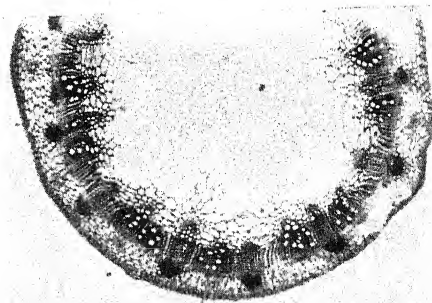
*b*



*m v*



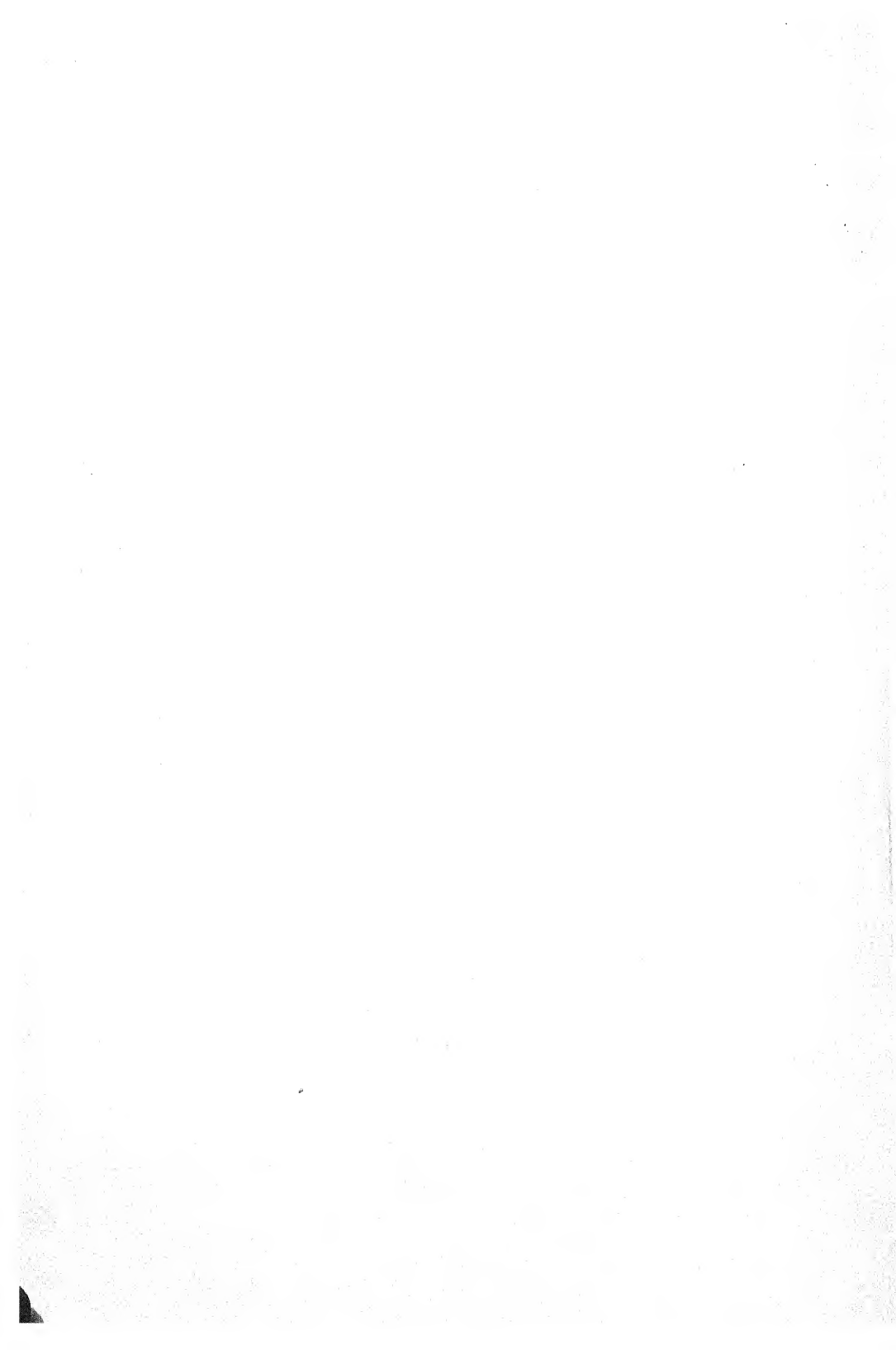
*r*



*B*

*M*

PFEIFFER on PLANTS AND LIGHT





## ULTRA-VIOLET LIGHT PHOTOGRAPHY IN THE STUDY OF PLANT VIRUSES\*

FRANCIS O. HOLMES

(WITH PLATE IV AND ONE FIGURE)

It has seemed desirable for some time that photography with ultra-violet light, using a quartz-lens microscope, should be applied to the study of the infective juices of the virus diseases of plants. It was realized before the present work was undertaken that there were many difficulties which might make it impossible to obtain successful results. There existed the chance, however, that some one of the viruses available might consist of formed particles just too small to be seen with the visual microscope. If these particles were not too small and should possess a favorable refractive index, their images might appear on photographic plates exposed with light of short-wave length. The careful examination of these plates at leisure might make it possible to recognize structures characteristic of the virus-containing samples.

In order that this method of observation of the plants infected with a number of viruses might be thoroughly tested, arrangements were made with the Botany Department of Columbia University, through the kindness of Dr. R. A. HARPER, whereby their Zeiss ultra-violet light photomicrographic apparatus was made available to the Boyce Thompson Institute for Plant Research for a period of two years. It was hoped that a fairly complete preliminary survey of this field of investigation could be made in that length of time. The purpose of this paper is to report the methods used, the types of viruses studied by this means, and the results.

### Ultra-violet light photomicrographic apparatus

The apparatus used in the pursuit of these studies is shown in fig. 1. It was devised by KÖHLER, who described it thoroughly in

\* Contribution from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York.

a long paper.<sup>2</sup> STEMPELL<sup>3</sup> used this apparatus in his research which resulted in the photographing of the spiral filaments of *Nosema bombycis* within the spore. The apparatus is constructed to allow the sorting out of light of low-wave length from the cadmium spark, and the isolation of a relatively pure beam of light corresponding to the wave length of 275 millimicrons. This light is led through a

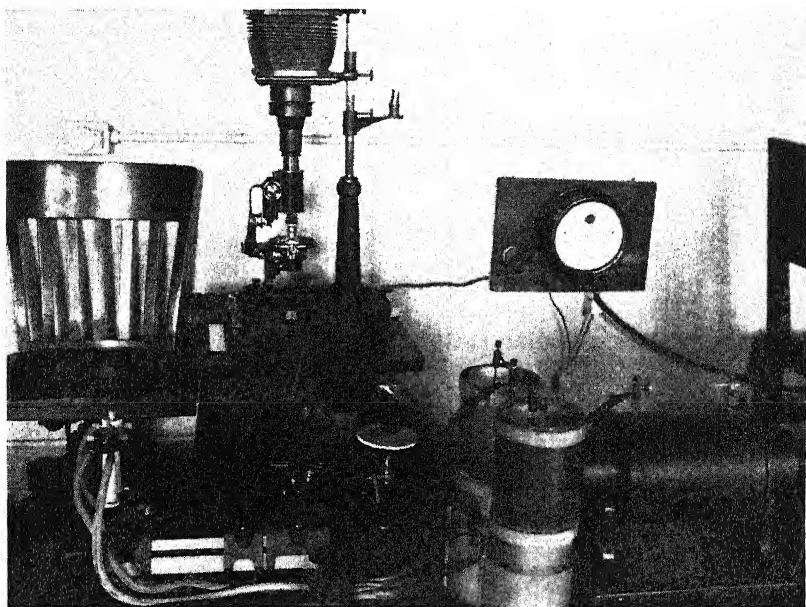


FIG. 1.—Zeiss apparatus for ultra-violet light photography: light obtained from a spark between cadmium or magnesium electrodes; quartz lenses and prisms isolate light of desired wave lengths and conduct it to microscope, which is equipped with quartz lenses.

series of quartz prisms, and passed through the microscope, which is provided with excellent quartz lenses adjusted for this exact wave length. Since the light is monochromatic, no chromatic aberration interferes with the sharpness of images secured. Objects to be photographed must be unstained and mounted between quartz slides and cover slips to insure complete transmission of the ultra-violet light.

<sup>2</sup> KÖHLER, A., Mikrophotographische Untersuchungen mit ultravioletttem Licht. Zeitsch. Wissen. Mikr. 21:148, 273. 1904.

<sup>3</sup> STEMPELL, W., *Nosema bombycis*. Archiv für Protistenkunde 16:281-358. 1909.

The virus-containing juices used were quite satisfactory, in that they contained just enough débris to make it certain that focusing on the several layers of a given mount was accurate, and that suitable small objects within the range of the instrument were being depicted.

### Typical viruses examined

The viruses to be studied were chosen with care to represent as varied types as possible. They were the following: aster yellows, tobacco mosaic, tobacco ring spot, potato witches' broom, potato leaf roll, potato rugose mosaic, potato aucuba mosaic. The aster yellows material was available at the Institute, where insect transmission experiments were under way. This virus causes chlorosis and the development of secondary buds, but no mottling or necrosis on the leaves. It is not mechanically transmissible. In complete contrast to this disease were tobacco mosaic and tobacco ring spot. The mosaic causes intense mottling with profound distortion of leaves, but with no necrosis; ring spot causes a necrotic pattern of concentric rings, but produces little chlorosis. Both of these tobacco viruses are easily transmitted mechanically, in contrast to the aster yellows virus which resists all attempts at transfer by rubbing or related methods.

On potato, additional types were made available through the kindness of E. S. SCHULTZ of the U.S. Department of Agriculture. The first of these was witches' broom, which is not related to aster yellows, but which causes growth of buds normally remaining dormant, thus simulating the appearance of aster yellows plants with less yellowing of the leaves. The second virus from potato was leaf roll, differing from all those thus far mentioned in causing no strong chlorotic or necrotic pattern on the leaf surfaces, but distinguished by a characteristic roll of the leaves. The third of this group was potato rugose mosaic, a mosaic comparable with the strong mosaic of tobacco in that it is readily transmitted mechanically. The fourth and last of the potato juices photographed was that containing the virus of aucuba mosaic, which causes isolated circular chlorotic spots on the foliage, but causes no noticeable leaf distortion or crinkling.

To collect a more varied group of viruses than these would be

difficult. It was necessary to have as representative a group as possible without introducing too many individual viruses, because the labor involved in properly studying a single additional type is considerable. It was hoped that these viruses might differ among themselves fundamentally, and that if size of the infective agent was the factor which has made it impossible to discover what viruses really are, some one of the seven might prove favorable in this respect, and furnish structures within the size range of the ultra-violet light instrument.

The juices to be photographed were obtained from the petioles of leaves of affected plants by pressing these against the quartz slide to secure a drop of fluid together with some débris from the broken cells. Such fluids in the case of tobacco mosaic are capable of causing the disease when introduced into healthy plants. The fluid was mounted for photographing between a quartz slide and a small quartz cover slip, and sealed in place with a ring of vaseline. The whole mount was placed at once under the microscope. A rough focus was secured with daylight by placing in the field of vision an air bubble purposely admitted into the preparation. This air bubble was still visible when ultra-violet light was passed through the preparation and caught on the fluorescent finder provided for the purpose. It could then be moved by means of the mechanical stage to a position at the edge of the field where it would appear in pictures taken at low magnifications, but not in the restricted fields taken by the more powerful quartz oculars.

Following the more careful adjustment of the focus by means of the fluorescent finder, a series of strip pictures was taken with a low power quartz ocular, the fine adjustment setting being recorded at each exposure. The plate thus secured was developed at once. With the best focus established, work began in earnest. The low power ocular was replaced by a higher. No inaccuracy was introduced in this way, as the lenses were made well enough to be in absolute agreement in giving a sharp focus. The same quartz objective was used throughout. A series of pictures was then taken, two on each plate, beginning with a point just below the correct focus and continuing to a point just above this focus. This allowed

a series of pictures to be examined for each spot of virus-containing juice photographed. It was found desirable to have at least a little *débris* from the plant tissues in each field to make possible an accurate comparison of the different levels, and to give assurance that delineation was satisfactory under the working conditions from day to day.

Difficulties were met in keeping the light intensity uniformly high, since a very slight change of direction of the beam causes a decrease in the illumination of part of the field at least. With light from the cadmium spark passing obliquely through a small eccentric stop and the highest objective, no decrease in intensity is permissible. Vibrations were eliminated as far as possible. The sealing of the plant juice in the mount and its subsequent exposure to ultra-violet light might seem to endanger the virus, yet motile bacteria handled similarly did not seem to be harmed, preparations after photographing appearing as fresh and as active as before.

It may be well to consider the conditions under which ultra-violet light photographs would be unable to depict small objects. If all the units to be studied were in active motion, whether showing Brownian movement or independent activity, no satisfactory images could be obtained. Among the most actively swarming bacteria, however, there are always some at rest on the surface of the cover slip, and the same is true of particles most of which may be in Brownian movement. If the refractive index were like that of the surrounding medium, and the absorption of ultra-violet light were low, particles would fail to impress their images on the photographic plate. The absorption of light by very small objects is in fact quite low. Small bacteria do not hold back much light, but because of their refractive indices they make distinct images on the photographic plate. The refractive index is sufficiently high in all known organisms to allow of photographing, but it might conceivably be low in the viruses. If this should be the case it might be impossible to see or photograph them. If, again, the causative agent were an organized structure smaller than 70 millimicrons in diameter, it would be hopeless to attempt to depict it with the type of short wave length apparatus now available. It would then be necessary

to have a powerful source of light of shorter wave length than 275 millimicrons to succeed.

In this investigation the writer was concerned only with the range between the limit of resolution of the visual microscope, with particles in the neighborhood of 150 millimicrons in diameter, and the limit of the ultra-violet light instrument with oblique light with particles approximately 75-80 millimicrons in diameter. The results obtained from the whole study were negative. In the virus-containing juices of the seven diseases investigated, no formed particles were observed to differ from those depicted in the juices from corresponding healthy plants. A careful search was made through the extensive series of negatives obtained during two winters' work, but no structures characteristic of the infective juices could be detected.

About 600 photographs were made of the virus-containing juices. In addition a series of photographs of known bacterial plant pathogens was made for comparison. This series included *Bacterium tumefaciens*, *Bacillus amylovorus*, *B. caratovor*, *Bact. campestre*, and *B. melonis*. The species were all photographed without staining, using living organisms. The results show that the ultra-violet light photomicrographic apparatus is of value in the study of the precise morphology of living, unstained bacterial organisms. It is already well known to be suited to the study of minute fungi and algae. KÖHLER and KRUIS<sup>4</sup> have published photographs of a number of species of living bacteria photographed in this way with ultra-violet light without the use of stains, but as the species were mostly unfamiliar, and KRUIS's publication is very difficult to secure, the five species of plant pathogens just mentioned are shown in an accompanying plate.

### Summary

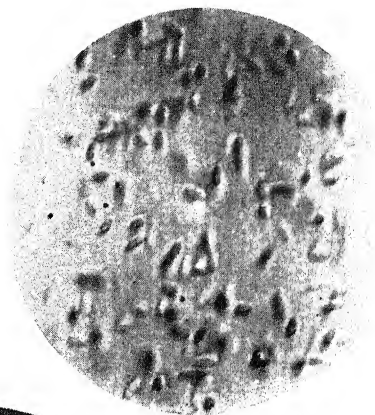
1. Seven typical juices from plants affected with virus diseases were photographed with ultra-violet light of wave length 275 millimicrons, but no formed structures other than those seen in corresponding fluids from healthy plants were found.

<sup>4</sup> KRUIS, K., Rozprawy české akademie císaře Františka Josefa pro vědy, solvesnost a umění. Trída 2, R. 22:23. 1913. (Bacteria photographed with ultra-violet light.)





1



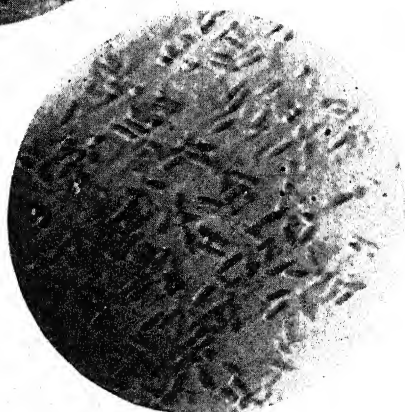
2



3



4



5

HOLMES on PLANT VIRUSES





2. The plant juices photographed were from asters having aster yellows, tobacco infected with tobacco mosaic and tobacco ring spot, potatoes having witches' broom, leaf roll, rugose mosaic, and aucuba mosaic.

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#### EXPLANATION OF PLATE IV

All the figures are of unstained living bacteria,  $\times 2000$ .

FIG. 1.—*Bacillus caratovorus*.

FIG. 2.—*Bacillus amylovorus*.

FIG. 3.—*Bacillus melonis*.

FIG. 4.—*Bacterium tumefaciens*.

FIG. 5.—*Bacterium campestre*.

## CYTOLOGICAL STUDY OF THE INTRACELLULAR BODY CHARACTERISTIC OF HIPPEASTRUM MOSAIC<sup>1</sup>

FRANCIS O. HOLMES

(WITH PLATE III)

The intracellular bodies characteristic of corn mosaic, sugar cane mosaic, tobacco mosaic, *Hippeastrum* mosaic, and related mosaic diseases have been studied by a number of investigators. Some have suggested that the bodies represent living organisms. The general appearance of the bodies is that of vacuolated cytoplasm, and their close association with the affected areas of the host plants makes the hypothesis seem reasonable. The lack of any visible nucleus in the mass has been the most important argument against the supposition that the body represents some stage in a parasitic organism. In the absence of any substitute for this criterion, the opposing idea that the body represents a waste material or distorted cell constituent has gained a large following.

IWANOWSKI (7) in 1903 described the bodies in tobacco mosaic, giving an almost complete description of them as they are known today, but interpreting their granular structure as proof that they were masses of bacteria. KUNKEL (8) in 1921 described and pictured the inclusions characteristic of corn mosaic, enumerating the ways in which the bodies resemble amoeboid protozoa, and pointing out the lack of characteristic nuclei. He later published accounts (9, 10) of the similar intracellular inclusions in *Hippeastrum* mosaic, sugar cane mosaic, and the mosaic of Chinese cabbage and tobacco. GOLDSTEIN (2) and RAWLINS and JOHNSON (11) described the appearance of the inclusions in mosaic tobacco. SMITH (12) studied a number of infectious and non-infectious chloroses, and found that the vacuolate bodies occurred only in the infectious type. HOGGAN (6) recently examined many hosts of tobacco mosaic, and found intracellular bodies consistently in all hosts which showed characteristic macroscopic

<sup>1</sup> Contribution from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York.

symptoms. She found no such bodies in the same hosts when affected with cucumber mosaic. GOLDSTEIN (3, 4) made extensive studies of the intracellular bodies of tobacco and dahlia, and reported the presence of the bodies in dividing cells, and the division of the bodies there with subsequent distribution by the division of the host cells.

The object of the present paper is to set forth the results of the writer's study of the inclusion bodies which occur in *Hippeastrum* mosaic. The work has been done from the protozoological viewpoint, treating the structure provisionally as though it were a protozoan, the exact systematic position of which was in doubt. It has not been possible to prove whether the structure is or is not a parasitic organism. It has been possible, however, to carry out studies which furnish data not before available. A careful search for nuclei and nuclear material within the body, and an equally careful study of the chondriosome content of the body have been made.

### Materials

Almost all of the work herein described was done on a mosaic infected stock of the species *Hippeastrum equestre* (Herb.), which was available at the Institute. Since it proved to be impossible to secure seed from this stock because of clonal sterility, and since no healthy plants were at hand, it was necessary to carry out a part of the investigation on a stock of seedlings obtained from hybrid plants closely resembling this species. The seedlings raised in the greenhouse were invariably free of mosaic, whether from apparently healthy or from the most severely infected parents. They remained completely healthy so long as they were retained in the greenhouse. By placing a portion of the lot of plants in the Institute garden during the summer, with mosaic plants in the same row, enough transmission occurred to give an additional stock of diseased plants.

*Hippeastrum* was used as a source of mosaic intracellular bodies for this investigation because the bodies in this plant are very large and very numerous, resembling in a general way those of corn and sugar cane.

### Search for nucleus in intracellular body

Keeping in mind the hypothesis that the intracellular body might be a parasitic organism, the search for a nucleus or nuclear material

was carried on in every way which seemed likely to lead to success. It was recognized as a general principle that the greatest variety of fixing agents and cytological stains present practically the same picture so long as they act on the same structures; but it was thought worth while to test the typical groups of fixers and stains in the hope that if nuclear materials were present, they might be recognized more easily in the shades of differentiation resulting from the various processes.

The following typical fixing solutions were used: Schaudinn's fluid (a mercuric chloride-fixing agent); Flemming's weak solution (an osmic acid-fixing agent); Bouin's fluid (a formalin picric acid-fixing agent); Carnoy's fluid (an absolute alcohol mixture); and Regaud's fluid (a formalin potassium dichromate-fixing agent). It was felt that these represented the typical fixers needed to insure enough variation in appearance of any structures which might be present in the bodies. The stains used were as follows: iron haematoxylin, destained generally with iron alum; Giemsa's stain, unmodified, staining nuclear material ruby red; acid fuchsin, long stain, destained in tap water; Flemming's triple stain. It will not be necessary in this paper to describe the methods of preparation and use of the fixing agents. For formulas for all of them, and a description of the staining methods, LEE's *Microtometist's Vade Mecum* may be consulted.

In addition to the cytological methods indicated, photography with monochromatic blue light was used to study the general ground material of the body. This light was of wave length 448.1 millimicrons, and was derived from the spark between magnesium electrodes. Many of the blue light photographs, such as are shown in the accompanying plate, show the extremely granular appearance of the bodies. The substance of the intracellular body is frequently very conspicuously granular, even when viewed in unfixed, unstained material. These photographs also show the general contours of the bodies as seen in section. Most of the pictures represent bodies from epidermal cells where no chloroplasts are present. Thus only the cell walls, the cell nuclei, and the inclusion bodies appear. In cells below the epidermis the chloroplasts are often conspicuous, and may be in close contact with the inclusion.

The inclusion bodies react in the same way as the cytoplasm of a young cell does to all types of fixing solutions. This is surprising in view of the fact that they are within the cytoplasm of relatively old host cells. Fixing agents preserve more of their substance than is the case in the nearby fluid cell cytoplasm. Their contours are consequently more easily distinguished in stained preparations than are the contours of the enveloping cytoplasmic strands.

In the search for nuclear material in the inclusion bodies, only two types of structures were found which could possibly be interpreted as nuclei. In most lots of material from mosaic plants the bodies were found to possess a few granules capable of retaining stains intensely. When present, these granules were retained with all the fixing agents used. They stained intensely black with iron haematoxylin, purple with Flemming's triple stain, and brilliant red with acid fuchsin, as do protein granules in many plant cells. They were not observed in material stained with Giemsa's stain, but presumably took a blue stain if they were present, since they were not apparent against the uniform light blue of the intracellular body as stained by this method. Such granules as these are present in the cytoplasm of the *Hippeastrum* plant, and may perhaps represent stored food material. In the absence of any other characteristic than their ability to retain most dyes intensely, they cannot be said to resemble nuclear material very strikingly. Particularly is their absence from material stained with Giemsa's stain an argument against their nuclear nature, as will be mentioned presently. These brightly staining granules have been observed in the intracellular bodies of other mosaic diseases (8).

Giemsa's stain is capable of giving a very intense blackish red stain on nuclear chromatin, and a large series of sections of mosaic *Hippeastrum* was therefore prepared with this stain. The stain reacted perfectly in the immediate neighborhood of the intracellular body. Thus the host cell nucleus was so characteristically colored that even the smallest fragment of it was recognizable. The intracellular bodies were immediately adjacent to the well stained plant nuclei, but no nuclear material was shown in them. They stained uniformly light blue, like cytoplasm. It is believed that this Giemsa stain test is evidence that the bodies do not contain nuclear material.

The other nucleus-like structure within the inclusion body is one not previously described. It has the appearance of a sphere or spheroid, possessing one and occasionally two deeply staining balls of material located peripherally. Such spheres may occur singly, or there may be as many as a dozen within a single body. In diameter the spheres range from 0.75 to 2  $\mu$ . When present in numbers within a body they are not clumped, but well distributed through the mass. Besides being found in the intracellular bodies, these spheres are sometimes present in the cytoplasm of the host cell. In this location they are clumped in some cases, in others well distributed. They may be scattered between chloroplasts in cells containing such plastids.

Healthy seedlings have never shown such spheres in their cells, although search has been made in more than 50 plants. In plants grown in the field long enough to show slight traces of mosaic infection, these spheres have been found occasionally. In heavily diseased plants the spheres may be present in considerable numbers or entirely absent. Material fixed with Carnoy's fluid, Flemming's weak solution, and Regaud's fluid has been found to retain the structures. It happens that no material fixed with Bouin's fluid or Schaudinn's fluid has ever shown the spheres, but it is entirely possible that this is because too few lots were examined. With most dyes the spheres tend to take a surface stain, but the interior may sometimes be tinted. With iron haematoxylin and eosin the spheres may be outlined in black, or may be uniformly stained with eosin, the peripheral ball being deeply stained with the black of the iron haematoxylin in either case. With acid fuchsin, which stains ordinary nuclei brownish red and nucleoli brilliant red, the spheres are dull red and the peripheral balls brilliant red. With Flemming's triple stain the peripheral balls of intensely staining material become pink, the spheres being outlined in purple. With Giemsa's stain, which stains chromatin ruby red and nucleoli pale blue, the peripheral balls stain deep blue and the spheres stain in outline pale blue.

It is not certain what the nature of these structures is. Since they are the only conspicuous inclusion within the inclusion bodies themselves, an understanding of them may help to solve the ques-



tion of the nature of the intracellular bodies. Somewhat similar structures have been described in the cytoplasm of nerve cells in rabies by GOODPASTURE (5), and intranuclearly in human smallpox by CALKINS (1).

#### Chondriosome content of intracellular body

By a study of the chondriosome content of the body it was hoped that light could be thrown on some of the suggestions as to its nature. The numerous hypotheses advanced in the past to explain the intracellular body may be classified into four groups. (1) The body would be expected to have no chondriosomes in its mass if it were an abnormal chloroplast, leucoplast, chromoplast, or elaioplast, normal or abnormal nuclear material, a colony of virus particles free of host cytoplasm, or an abnormal tannin vesicle. (2) It would be expected to contain a moderate number of chondriosomes if it were a parasitic organism, living host cell cytoplasm containing virus, or living host cell cytoplasm without immediate contact with virus. (3) It might be expected to vary in chondriosome content if it were dying host cell cytoplasm or dead cytoplasm. (4) It might be expected to be completely composed of chondriosome-like material if it were a pile of chondriosomes aggregated, or fused, and perhaps chemically changing.

A typical lot of mosaic *Hippeastrum* tissue was fixed in Regaud's fluid, sectioned and stained with a long iron haematoxylin method. The chondriosome content of the cells and of the intracellular bodies was examined in a set of 100 slides.

The preparations consistently pointed to a definite conclusion. The intracellular bodies showed a moderate number of chondriosomes well distributed through their substance, just as the host cell cytoplasm nearby showed its expected quota, also well distributed. A group of photographs of a single intracellular body at different optical levels is shown in fig. 1, to demonstrate the uniformity of distribution of the chondriosomes in the mass of the body. The presence of a moderate number of well distributed chondriosomes in such a mass of material, in itself reacting like cytoplasm to all biological stains applied, is strong evidence for the view that the mass is

partly or wholly living cytoplasm, and is evidence against the opposing hypotheses which have been suggested. Of the possibilities, the three which are not opposed by this finding are: (1) that the body is a stage in the life cycle of a parasitic organism causing the disease; (2) that the body is a mass of host cell cytoplasm containing virus; or (3) that the body is a mass of host cell cytoplasm not immediately associated with virus, although perhaps holding its form because of the stimulation given by the diseased condition.

The hypotheses opposed by this finding of a normal number of well distributed chondriosomes within the intracellular body are also opposed individually by many small bits of evidence. Thus, for example, the probability that the body is not an abnormal elaioplast is indicated further by the fact that normal elaioplasts are found in the stems of mosaic and healthy plants alike, but no intergrading forms occur. Between the three possibilities not opposed by the chondriosome study no choice can be made at present, for no evidence is known to favor any one of them decisively.

### Summary

1. A cytological study of the intracellular bodies characteristic of *Hippeastrum* mosaic disclosed the fact that no nuclear material is to be found in the mass of the body, unless two types of structures should be so interpreted. These are (1) intensely staining dots not markedly different from others found outside the body in the fluid cytoplasm of the host cell; and (2) spheres containing deep-staining, peripheral, single or rarely double balls. These spheres are very definitely formed and easy to recognize. They were found also in the host cell cytoplasm in diseased plants, but not in that of healthy plants. These are the only formed structures of distinctive appearance within the intracellular bodies associated with *Hippeastrum* mosaic. It has not been possible to identify them.

2. Chondriosomes were found within the intracellular bodies in moderate numbers, well distributed through the mass. This observation is considered evidence for the view that the intracellular body in this particular disease consists of living cytoplasm. Whether the body represents a stage in a foreign organism, a mass of plant cell

cytoplasm containing virus, or a mass of the plant cell cytoplasm not immediately in contact with virus but stimulated by the diseased condition, is not known.

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#### EXPLANATION OF PLATE III

All photographs were taken with blue light from the magnesium arc, wave length 448.1 millimicrons.

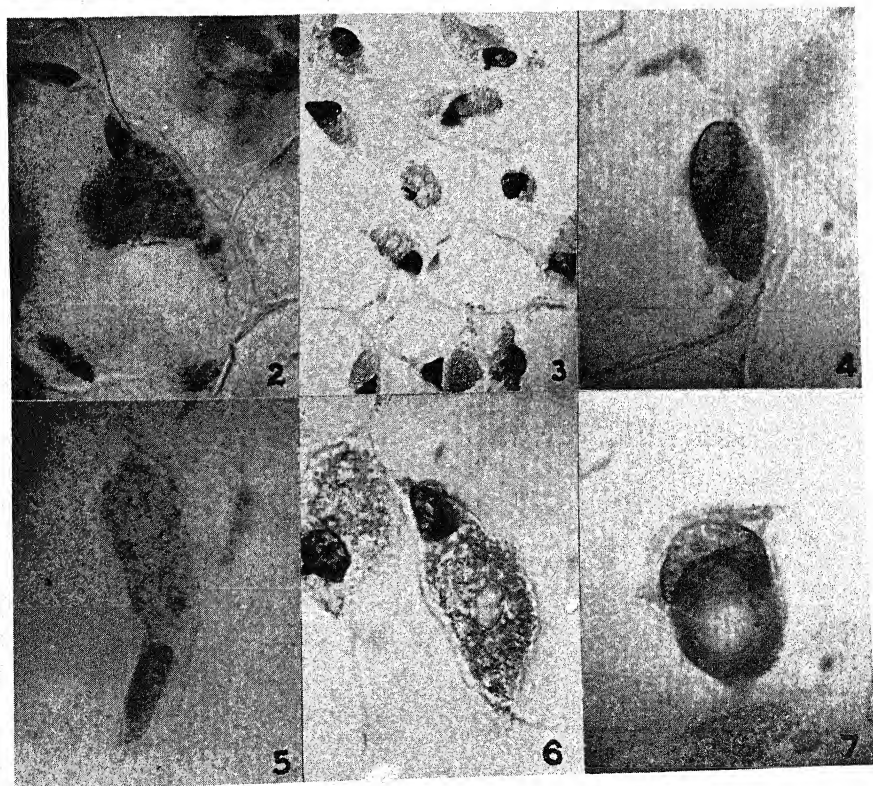
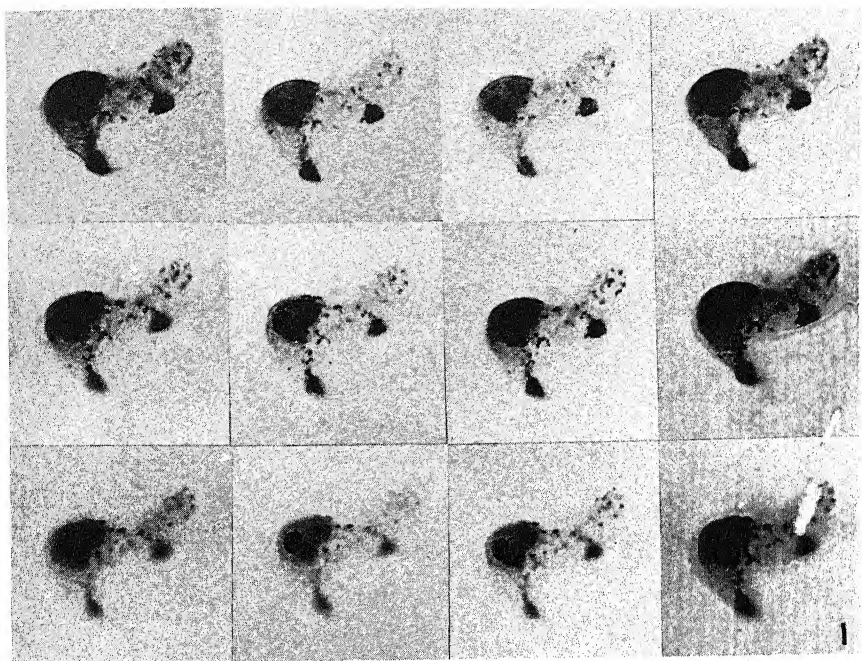
FIG. 1.—Nucleus and adjacent intracellular body photographed at twelve different optical levels to show that the dotlike chondriosomes are distributed through mass of body and not merely on its surface; from mosaic *Hippeastrum* tissue fixed in Regaud's fluid and stained with iron haematoxylin; nucleus at

left, intracellular body containing well distributed chondriosomes at right; two plastids below nucleus and body against wall of cell;  $\times 1000$ .

FIG. 2.—Intracellular body containing five spheres, only one showing in photograph; peripherally located deeply staining dot can be distinguished in sphere-like structure;  $\times 1000$ .

FIG. 3.—Low power photograph of section through epidermis of mosaic *Hippeastrum* plant; nuclei of cells appear very dark, bodies vacuolated and granular; fixed in Schaudinn's fluid, stained with Giemsa's stain;  $\times 300$ .

FIGS. 4-7.—Intracellular bodies showing granular structure, especially in fig. 6, vacuoles in figs. 6 and 7, close contact with nucleus in all; figs. 4, 5, and 7 from material stained with acid fuchsin following Bouin fixation; fig. 6 from material fixed in Schaudinn's fluid and stained with Giemsa's stain;  $\times 1000$ .



HOLMSIA on HIPPEASTRUM



## ACCURACY IN QUANTITATIVE WORK WITH TOBACCO MOSAIC VIRUS<sup>1</sup>

FRANCIS O. HOLMES

(WITH THREE FIGURES)

It is often desirable, in working with the readily transferred virus of tobacco mosaic, to have some means of telling whether one sample of the infective juice is more infectious or less so than another. This has frequently been accomplished by inoculating from ten to fifty plants in one of the accepted ways of mechanically transmitting the disease, and then judging from the number of resulting infections which of the two sources contained the larger proportion of virus in a given volume. The accuracy of this procedure is sufficient to allow a correct differentiation to be made between strong and weak samples of virus. Samples differing but slightly in strength can be differentiated and properly graded only by inoculating larger numbers of plants.

A recent paper by MCKINNEY<sup>2</sup> recommended a standard procedure for quantitative studies. He emphasized the difficulty of growing large enough numbers of plants in the greenhouse space usually available. The methods described in the present paper allow quantitative work to be done with economy of space, convenience, and rapidity of manipulation while inoculating, and a high degree of accuracy. It is particularly important to know what accuracy may be expected in experiments with viruses of known relative concentrations. With this information in hand the numbers of plants needed for proposed studies may be calculated.

During the study of plant disease viruses by means of ultra-violet light photography, it was desired to know how many units of virus, if such exist, were to be found in the small volumes of

<sup>1</sup> Contribution from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York.

<sup>2</sup> MCKINNEY, H. H., Quantitative and purification methods in virus studies. Jour. Agric. Research 35:13-38. 1927.



material represented in the individual photographs. For this reason the methods described in this paper were worked out. It was later found possible to obtain data from which the accuracy of such quantitative determinations of virus strength could be calculated, and from which the numbers of plants required to perform given inoculation experiments could be estimated in advance.

### Procedure in making measurements

Since large numbers of inoculations were to be made, in order to reduce the effects of chance errors, it was evident that some uniform but very rapid method of performing the inoculations would

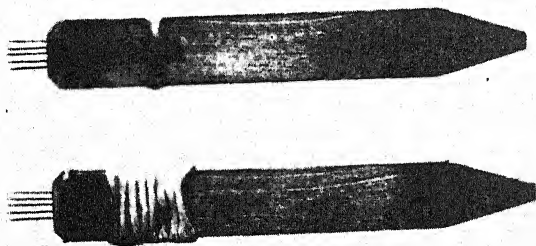


FIG. 1.—Inoculating needles, described in text

be needed. Punctures made with black enamel insect pins, size no. 00, were found to produce wounds of exceedingly regular size. Through these pin pricks tobacco mosaic was readily transferred, merely by alternate punctures in leaves containing virus and in leaves of healthy test plants. The pins used were obtained fresh for each experiment, so that no contaminations occurred because of the inoculating instruments. The fine points of the pins penetrate the leaves with the slightest pressure. It is not necessary to touch the plants at any time with fingers or instruments other than the insect pins actually used to introduce the inoculum. The uniformity of the dose lifted on the pins and transferred to new plants from mosaic specimens or from extracted juice is evident from the results. To obtain a fair percentage of infections it is necessary to

make about five pin pricks in each test plant. The work of transfer is therefore very greatly facilitated by using, instead of a single insect pin, a set of five pins bound in a temporary handle between two wooden pot labels (fig. 1). Rapidity of inoculation and uniformity of dose being thus combined, it is possible to make the required inoculations at the rate of 500 or more an hour. This method was found to be very satisfactory, and allowed many interesting experiments to be performed. Greenhouse space must be conserved if large numbers of determinations are to be made. The use of the simple inoculating apparatus just described makes it possible to utilize small tobacco plants. The wounds made by the pin prick inoculations into the leaf tissue are so small and heal so readily that even young plants are not held back by the introduction of the inoculum. No dead tissue appears around the site of the inoculation, as happens sometimes with rougher methods. It has been suggested that such dead tissue may hold back part of the virus applied and prevent its entry, so that the clean healing under the circumstances seems favorable.

In all of the experiments described in this paper, Turkish tobacco was used, because of the desirable shape of the plant for greenhouse studies, and because of its marked susceptibility to the mosaic disease. Its upright habit of growth and short leaves economize space. The tobacco plants were transferred to flats when they had a leaf spread of about one inch, and when well established were used for inoculation. The results can often be obtained before serious crowding begins, but crowding never caused trouble by the accidental transfer of the disease. Judicious care in handling plants and freedom from insects allow tobacco mosaic experiments to proceed with practically no contaminations.

In fig. 2 are shown the types of plant used. The wooden flat at the left contains small plants which have just been transplanted to it. The middle flat shows plants established and ready for inoculation. The flat at the right contains plants already diseased and ready to be discarded. So many plants can be placed in a greenhouse bench, when they are thus planted in wooden flats, that the space requirement for performing extensive experiments in a quantitative way is reduced to a minimum.

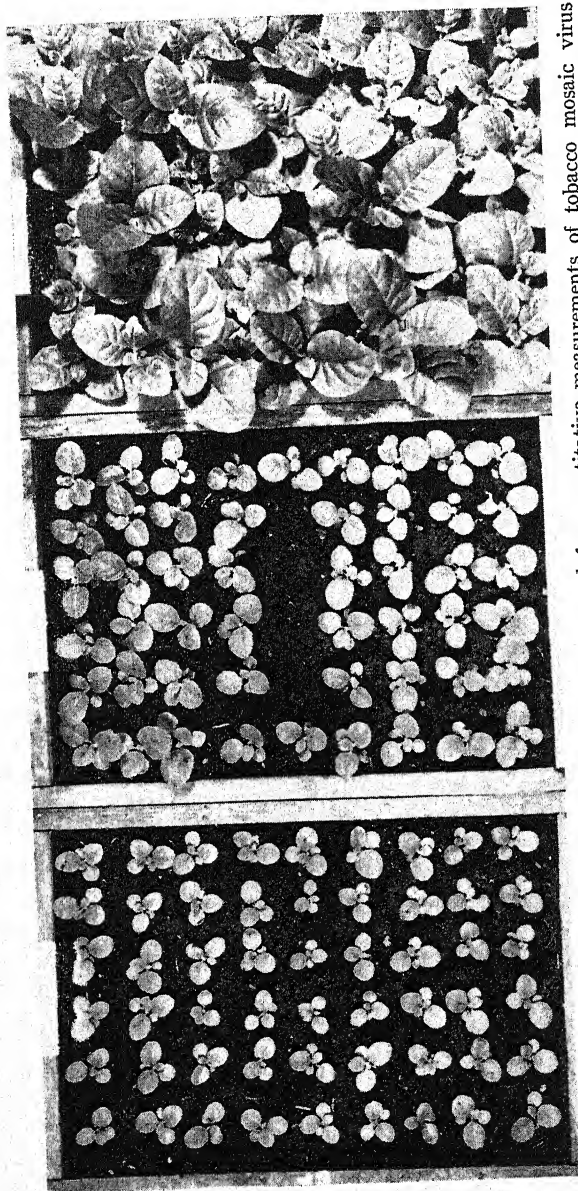


FIG. 2.—Turkish tobacco plants in wooden flats as used for quantitative measurements of tobacco mosaic virus strengths: at left, plants just transferred to flat; center, plants a week later when well established and ready for inoculation; at right, plants after results of inoculation are recorded, flat ready to be discarded.

## Results in measuring known samples

In order to know the errors involved in measuring the strengths of virus samples, experiments with definite strengths of virus were needed. A stock virus was therefore made by cutting 100 or more mosaic plants of Turkish tobacco into small pieces and expressing their juices into a glass bottle. The actual strength of this stock virus at any one moment was not known, but by measuring out one portion of this virus and mixing with it three portions of water, it is clear that a new sample of the virus is obtained, the total virus content of which, in a given volume, is one-fourth that of the original

TABLE I\*  
REDUCTION IN NUMBER OF INFECTIONS IN EACH SET OF 50 TEST PLANTS  
THROUGH USE OF KNOWN DILUTION

COMPARISON OF UNDILUTED VIRUS WITH	MEAN REDUCTION IN EACH SET OF 50 TEST PLANTS	P.E. SING.	NO. OF SETS COMPARED WITH UNDILUTED VIRUS	P.E. MEAN	STANDARD DEVIATION
1:2.....	3.7	4.5	20	1.0	6.5
1:4.....	7.5	4.6	24	0.9	6.7
1:8.....	11.2	3.3	16	0.8	4.8
1:16.....	15.3	5.2	16	1.3	7.4
1:64.....	22.4	2.2	8	0.8	3.1

\* For compactness in this table the mean reductions are given. As an example of the original figures obtained in the experiments, there follow the paired observations on undiluted and diluted samples from which the values shown in the 1:8 item above were calculated: 35-29, 29-8, 36-28, 28-12, 22-11, 11-4, 20-11, 11-3, 42-29, 29-19, 44-27, 27-21, 44-27, 27-20, 34-28, 28-11. It will be noted that the reduction on dilution is independent of the strength of the undiluted sample, as is implied by the graph shown for these values in fig. 3.

sample. As will be shown later, this new sample upon inoculation into test plants does not give a reading one-fourth that given by the original sample, although it contains only one-fourth as much virus per unit volume. The effectiveness of a dilution for purposes of inoculation is not reduced by the addition of water so rapidly as is the actual concentration.

By adding water to the stock virus in other proportions, other definite dilutions may be obtained. A considerable number of such dilutions were prepared and all were promptly used for inoculation. Fifty inoculations require exactly five minutes. No great deterioration would be expected in such short intervals, and the consistency of the readings indicates that deterioration did not interfere with the process. In measuring known dilutions 2500 plants were used.

When the results were tabulated, as shown in table I, it was found that dilution to one-half original strength causes on the average a drop of  $3.7 \pm 1.0$  infections per set of 50 plants inoculated. This drop is independent of the strength of the original sample over the range studied. A dilution to one-fourth results in a decrease to the

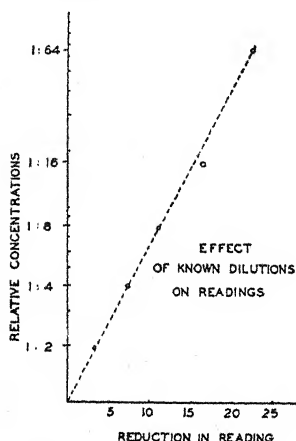


FIG. 3.—Graph showing effect of reducing virus strength by dilution; reduction in reading due to dilution is expressed in terms of average difference in number of infections resulting when undiluted and diluted samples are used, each in a set of 50 test plants.

extent of  $7.5 \pm 0.9$ ; a dilution to one-eighth causes a decrease of  $11.2 \pm 0.8$ ; the still greater dilution to one-sixteenth results in lowering the reading by  $15.3 \pm 1.3$ ; and finally the dilution to one-sixty-fourth of the original strength causes a decrease in the number of infections amounting to  $22.4 \pm 0.8$  plants. A graph from which intermediate points may be read is shown in fig. 3.

As will be noted from the graph, the character of this response to dilution is such that the original strength of the virus does not affect the numerical reduction in infections resulting from the dilution. The numerical reduction due to dilution is dependent upon the degree of dilution, but not upon the strength of the sample taken for dilution. This makes it possible to use the information contained in the graph without the necessity for obtaining a virus of standard strength. With this information in hand, it is possible to calculate at any time the number of plants required to perform a given experiment in which it may be desired to differentiate between two viruses of moderately different strengths, or in which it may be necessary to compare two viruses with accuracy. If no limit to the number of plants available existed, it would be possible to obtain any desired degree of accuracy. Future improvements in technique may make it possible to determine virus concentrations as accurately as bacterial numbers may be estimated or chemical concentrations may be calculated from quantitative analyses.

For the present it seems necessary to be satisfied with a degree of accuracy demanding only a few hundred seedling plants. Table II shows the number of plants required to determine with good odds for significance the order of concentration of samples of virus of various strengths. Thus to distinguish with fair certainty between an undiluted sample and one a sixty-fourth as strong, some 30 plants are needed for each of the two samples in the comparison. If the difference between the two samples is but one to sixteen, 60 plants are required. If the difference is one to eight, 120 plants will suffice. If one to four, 240 plants must be used. If one to two, 1350 plants

TABLE II

NUMBERS OF PLANTS REQUIRED TO DETECT MODERATE DILUTIONS

$$N = \left[ \frac{\text{C.O.} \times \text{P.E. sing.}}{\text{Allowable deviation}} \right]^2$$

Average of P.E. sing. of difference between two dilutions = 4.0 (see table I)\*  
 C.O. for odds of 100:1 = 3.9      C.O.  $\times$  P.E. sing. = 3.9  $\times$  4.0 = 15.6

To compare undiluted virus with	Numbers required for each dilution; to be compared with same number from undiluted sample
1:2.....	$(15.6)^2 \div (3)^2 = 27$ sets or 1350 plants
1:4.....	$(15.6)^2 \div (7)^2 = 4.8$ sets or 240 plants
1:8.....	$(15.6)^2 \div (10)^2 = 2.4$ sets or 120 plants
1:16.....	$(15.6)^2 \div (14)^2 = 1.2$ sets or 60 plants
1:64.....	$(15.6)^2 \div (20)^2 = 0.6$ sets or 30 plants

\* The probable errors of single observations as listed in table I are so nearly equal, and have in themselves so large a probable error, that in calculating the numbers of plants required for experiments their average is used.

will be essential for the high degree of certainty implied by the odds of 100:1 that the difference observed is significant. It seems worth while to use these large numbers of plants since the labor involved is not great. The greenhouse space demanded is moderate compared with that often used in inaccurately determining virus strengths on smaller numbers of large plants. The equipment needed for the experiments is simple, being new insect pins for each experiment and a few wooden pot labels.

The formula by means of which table II was constructed may be of interest to some, since it is necessary in determining for any new experiment the numbers of test plants to be used. It is:

$$N = \left[ \frac{\text{C.O.} \times \text{P.E. sing.}}{\text{Limiting deviation}} \right]^2$$

In this formula  $N$  stands for the number



of plants to be used in the experiment contemplated; C.O. signifies the coefficient of odds corresponding to the degree of certainty desired (see PEARL and MINER<sup>3</sup> for complete table; 3.9 may be inserted here in the formula if odds of 100:1 for a significant result will be satisfactory); P.E. sing. stands for the probable error of a single comparison; limiting deviation is to be replaced in the formula by the figure representing the allowable error beyond which the set of readings must not go in the experiment to be performed, lest the significance of the results be impaired.

### Some applications of the method

#### EFFECT OF STORAGE ON SAMPLES OF PLANT JUICE CONTAINING VIRUS

It is evident that, since the error of experiments can be known accurately and adjusted within desired limits, many interesting questions can be answered, using enough plants in each case to obtain trustworthy results. Viruses from different sources may be graded accurately according to their initial concentrations, methods of preserving viruses may be compared as to their efficiency, and treatments with chemicals may be interpreted in the light of their exact effects. One very interesting and practical problem concerns ways of storing virus so as to have as slow a change in strength as possible. The practical importance of the matter comes from the need for some method of holding a given virus from season to season in order that experiments made at different times may be comparable. For example, it is desirable to know whether susceptibility of host plants differs with the change from summer to winter growing conditions, as some have suspected. This problem cannot fully be solved until some fixed reference point, such as a stable stock of virus, has been secured. It has long been known that tobacco mosaic virus allowed to stand in a bottle in the laboratory remains infectious for years. The question has never been answered, however, as to how rapidly the virus may lose strength at first. Most observers have noted diminished potency after long standing.

A rather extensive experiment was performed as follows. A

<sup>3</sup> PEARL, R., and MINER, J. R., A table for estimating the probable significance of statistical constants. Maine Agric. Exp. Sta. Bull. 226. 1914.



quantity of fresh virus, amounting to some 300 cc., was divided into two lots. One-half was stored in a glass bottle on the laboratory desk at approximately 22° C.; the other half was stored in a room kept below the freezing point. Before the latter sample was frozen it was poured into tubes. One-half of these tubes were allowed to stand frozen, the other half were melted and refrozen ten times. Each time the virus was poured from tube to tube in such a way that the clear ice of high melting point was gradually separated from the brown mother liquor of low melting point, and this in turn from the green sediment which contained all the solid débris.

#### TESTS WITH VIRUS KEPT AT ROOM TEMPERATURE

The first test of the fresh virus was made at once upon the collection of the juice. Five flats, each of 50 plants, were used for a series of small dilutions of this juice, the same dilution series being used throughout the course of this whole experiment, so that the total readings from the series could be compared each time with other totals. As soon as this set of five flats had been inoculated, a second series exactly duplicating it was arranged. Thus inoculations on 500 plants, contained in ten flats, were made with the fresh virus. As will be seen in table III, which records the results of this experiment, the first series of five flats used for this fresh virus gave a total of 117 infections. The duplicate series gave 140. The average number of infections per set of 50 plants over the whole series was therefore 25.7 plants.

When the virus had stood at laboratory temperature for 24 hours a second similarly arranged test was made. Five hundred plants were again inoculated in exactly the same way, but with the one-day old virus. It was found that the number of infections had greatly changed, and that the two duplicate series agreed throughout in giving evidence of a decrease in strength. In five sets 50 infections had appeared. In the duplicate five sets 51 infections were found. The average number of infections per set of 50 plants was 10.1 plants, a much smaller result than was recorded for the fresh virus. If the fresh virus concentration be taken as 100 per cent, then it will be noted by reference to fig. 3, which represents the decrease due to dilution, that this one-day old virus acted as a 6

per cent suspension would be expected to do. If the reader desires to inquire into the accuracy of the duplicate series it will be possible to do so, using 4.0 for the probable error of a single comparison as shown in table II.

Before considering the behavior of this stored virus sample upon further aging, it must be mentioned that this experiment when repeated gives changed results according to the bacterial flora present and developing in the sample, and that this particular

TABLE III  
EFFECT OF AGING ON EXTRACTED VIRUS AT 22° C.\*

VIRUS	DUPLICATE DETERMINATIONS	OBSERVATIONS ON SERIES OF DILUTIONS					TOTALS	AVERAGES	STRENGTH INTERPRETED IN TERMS OF ORIGINAL (PERCENTAGE)
		Undiluted	1:4	1:8	1:16	1:64			
Fresh.....	{ 1....	35	29	29	16	8	117	25.7	100
	{ 2....	36	36	28	28	12	140		
One day old.....	{ 1....	22	11	11	2	4	50	10.1	6
	{ 2....	20	9	11	8	3	51		
One week old.....	{ 1....	42	41	29	35	19	166	33.0	400
	{ 2....	44	36	27	36	21	164		
Two weeks old....	{ 1....	44	43	27	22	20	156	29.7	210
	{ 2....	34	38	28	30	11	141		
One month old....	{ 1....	11	6	9	3	2	31	6.2	2
	{ 2....	11	7	5	3	6	31		

\* The individual readings were made on a set of 50 test plants each time.

case is being recorded to indicate the actual findings in one instance. It is on the whole a good illustration of what happens to a virus at room temperature. Following the inoculations at the end of the first day a bacterial fermentation took place in the virus sample. Bubbles of gas rose through the liquid, and an odor like that of a fresh hay infusion was noted. This fermentation may have broken up solid particles in the suspension, thus liberating virus. Whatever its mode of action, a change in virus concentration was very evident when inoculations were again made at the close of a week. The first five sets of 50 plants, each inoculated in this seven-day sample, gave altogether 166 infections. The accompanying series

of five additional sets gave 164 infections. The samples for the duplicate series were all prepared independently, no one being made from another, but all from the undiluted stock bottle by the process of pipetting out known quantities of the virus and of water. The average number of infections per set for this one week test was 33.0 plants. This corresponds to the reading which would have been given by a sample four times as strong as the original. At the end of another week, fourteen days from the time when the fresh virus was tested, a new examination was made of the state of preservation of the virus, and it was found that two readings, 156 and 141, corresponding to the totals given before, were obtained. The average number of infections per set of 50 plants was therefore 29.7 plants. This indicated a strength still double that of the original, but lower than had been recorded at the end of the first week.

At the end of one month of storage the sample was again tested in the same way. The first 250 test plants gave 31 takes, the second 21 takes. It appeared then that a rather low level had been reached, the average number of infections per set of 50 plants being 5.2, and the strength of virus indicated being approximately 2 per cent of the original concentration. A subsequent test after four months indicated that a slow decrease had continued to take place. It must be remembered that this loss of 98 per cent of the strength after some weeks of standing does not mean that the virus would be considered very weak under some conditions, for when heavy inoculations are made as a regular practice, it is customary to dilute fresh viruses with water to such an extent that much weaker suspensions than this are obtained. It is possible to have 100 per cent of infections result from the use of these weak samples, because a large quantity of the virus is applied to the cut surfaces of the leaves. In many experiments, however, the loss of a large part of the virus strength is to be avoided, and in such work the results of this experiment indicate that speed of operation is a desirable feature.

#### TESTS WITH FROZEN VIRUS

At the end of a month and a half the sample of the original virus which had been frozen was melted and tested. It appeared that it had not lost as much of its strength as had the room temperature

sample. It had lost approximately 85 per cent of its effectiveness, however, as judged by comparison with the dilution chart. In the test which determined this, the average number of infections per set of 50 plants throughout the same series of dilutions as was reported in table III for the room temperature sample was 15.2, a reading lower than that given in the earliest test for the fresh virus by 10.5 plants. It is this difference which indicates the amount of loss due to the long standing while frozen. It will be seen, therefore, that the virus stored at room temperature was reduced to 2 per cent of its original strength in a month; that which was stored frozen retained 15 per cent of its strength as long as one and a half months.

When tests were made on the frozen fractions, which had been secured by thawing and pouring in such a way that one appeared to be clear ice water, one a deep brown low melting-point solution, and the third a mass of green *débris* suspended in a little fluid, it was found that the bulky ice fraction and the smaller *débris*-containing fraction had lost very little more of their strength than had been lost by the whole virus. At the same time the brown fraction had increased significantly in strength. Apparently the virus had been frozen out of the clear ice to a slight extent, and had followed the pigments and dissolved salts into the small volume of the low melting point fraction. Further work will be necessary before an unchanging virus can be secured by any method of storage. These experiments, illustrating the extent of the changes which may take place when a virus stands in a glass receptacle for some weeks, at least indicate that a virus does not retain all of its strength over long periods of time.

#### GREEN AND YELLOW AREAS OF MOTTLED LEAVES

It is known that mottled leaves contain virus in the yellow areas and also in their apparently green areas. A careful examination of the green areas shows that they are sometimes invaded by yellowish patches, and that mistakes may arise in taking them all to be representative of the purest green areas available on green and yellow leaves. Preliminary tests showed that by loading the insect pin inoculating instruments with juice by sticking them

directly into tissues to be investigated and then into the leaves of healthy test plants, a great difference could be observed in yellow and green areas respectively as sources of virus. While the experiment recorded below does not show that the green areas are free from virus, it does show with certainty that the green areas are poor sources of virus as compared with the yellow areas immediately adjacent to them. In each of the ten parts of the experiment here described a single well marked leaf was taken, and examined first

TABLE IV  
GREEN VS. YELLOW AREAS OF SINGLE MOTTLED LEAVES AS  
VIRUS SOURCES

LEAF NO.	YELLOW AREA	GREEN AREA	DIFFERENCE IN READING	DEVIATION FROM MEAN	DEVIATION SQUARED
1.....	30	11	19	1.2	1.4
2.....	16	6	10	7.8	61.5
3.....	21	0	21	3.2	10.0
4.....	33	2	31	13.2	174.0
5.....	28	6	22	4.2	17.6
6.....	21	1	20	2.2	4.8
7.....	29	5	24	6.2	38.4
8.....	12	2	10	7.8	60.8
9.....	12	1	11	6.8	46.2
10.....	12	2	10	7.8	60.8
			Mean = 17.8	Sum = 476	

$$\sigma = \frac{476}{10} = 6.9$$

$$Z = \frac{17.8}{6.9} = 2.58$$

$n = 10$  Each reading was made on  
50 test plants.

Odds for significance 10,000:1; odds that yellow area is at least eight times as strong a source of virus as green area about 100:1.

by making transfers to 50 plants from the green areas, then by making similar transfers from yellow areas to an equal number of plants. The leaves were not chosen as comparable, but merely as having well marked patterns. The yellow area readings are not all of the same order of magnitude, as will be noted in table IV, where they are summarized, but the difference between the yellow area on a leaf and the green area on the same leaf is consistently large, and always in favor of the thin yellow tissue as the better source of infective material.

The number of infections from the yellow areas was 17.8 higher on the average per set of 50 test plants than the number of infections

from the green areas. By reference to the dilution chart it will be noted that this corresponds to the difference as virus source between an undiluted and a 1:28 virus. The odds that the average difference of 17.8 represents a real difference between the two sources are very high, approximately 10,000:1. It appears, therefore, that *in mottled leaves* the virus distribution is indicated by the yellow pattern. It is known that leaves below the mottled ones on a plant are not free of virus after the plant has been infected for a long time, even though they are green. They may contain virus in almost as great a concentration as that in the yellow portions of mottled leaves, yet some may be almost free of virus, depending on their previous history and their position on the plant, as will be seen from the following account.

#### SPREAD OF VIRUS IN INOCULATED PLANTS

By inoculating a series of similar plants near their growing points, and then examining their leaves from time to time by making large numbers of transfers to healthy test plants, it was possible to assemble the data summarized in table V. This table shows that the green leaves above the point of inoculation quickly built up a concentration of virus comparable with that found in the yellow areas of mottled leaves, but that the green leaves below the point of inoculation remained for many days free of virus, later possessing a considerable amount. From the time of the appearance of the earliest symptoms, it is evident from the large numbers of infections secured in surveying leaves above the point of inoculation, that virus was present in the upper part of the plant. Only the column marked "developing leaf" has to do with leaves which show clearing of veins or mottling. The inoculated leaves never showed symptoms, yet they came to contain large amounts of virus. It seems necessary to assume from these figures that the larger part of the virus present in these green leaves below the mottled ones, but above the point of inoculation, was formed in them, and was not due to a backward flow from the developing leaves, because the original point of inoculation marked the limit of the virus-containing leaves. If the virus should flow back down the plant in quantity, it would not be expected to stop at the inoculated leaf. Yet virus was found, from the

time of the appearance of the earliest symptoms until the end of three weeks from the time of inoculation, to be present in large amounts in the inoculated leaf and those above it, and to be absent from the leaves immediately below. The later development of virus in the lower leaves must indicate a migration of at least a small amount of virus backward down the plant.

TABLE V  
SURVEY FOR SPREAD OF VIRUS\*

	LOWEST GREEN LEAF	INTERMEDI- ATE LEAF (BELOW)	ORIGINALLY INOCULATED LEAF	INTERMEDI- ATE LEAF (ABOVE)	DEVELOPING LEAF
Inoculated when 8 inches high					
Examined at:					
Clearing of veins.....	0	0	29	43	42
First mottling.....	1	0	25	45	39
Three weeks.....	0	0	32	37	36
Four weeks.....	49	47	42	46	5
Five weeks.....	10	29	40	42	40
Inoculated when 1 inch high					
One week.....			11	30	24
Two weeks.....			28	38	42
Three weeks.....			35	48	43
Four weeks.....			45	39	43
Five weeks.....			33	44	44

\* Each number in the table here given represents the results of the inoculation of 50 test plants. The exact reading should not be considered important, since there is some variation when as few as 50 plants are used in each test. The groups of figures are consistent enough, however, to show the trend of virus concentration in the leaves in each position considered.

It seems necessary to conclude that the virus may develop and come to high concentration in leaves which appear very different, some being green, others mottled; and that mottling is not so much an indicator of the presence of virus as it is an indication that virus was present at the time the leaf developed. More careful studies will be made later. This survey indicates in a general way the behavior of the virus as it spreads through the plant. In addition to the points of theoretical interest involved, the survey throws some light on the length of time plants should stand infected before their leaves are used as sources of virus. It is hoped that the account here given of the method used for obtaining concentration readings on tobacco mosaic virus may be of value to other investigators who



would like to know the strengths of the viruses with which they may work, and what degree of reliance may be placed on readings of such strengths.

### Summary

1. A method of inoculating test plants with small, uniform doses of tobacco mosaic virus is described. The object of the method is to lessen the mechanical difficulties usually met with in the attempt to obtain reliable quantitative results. By the use of small plants, inoculated by pricks of insect pins held in a convenient handle, very large numbers of test inoculations may be performed with economy of time and effort, and with a minimum of greenhouse space and practically no danger of contamination from handling.

2. It is shown that dilution to a given extent causes a decrease in infections when inoculations are made into test plants, and that this decrease is not dependent upon the original concentration of the virus, but only upon the percentage dilution in the range studied. Charts and a graph are given so that the results with known dilutions may be available for grading unknown samples of virus. It is shown that the numbers of plants required for significant results in proposed experiments may be predicted.

3. Experiments are described showing that by the use of such methods it can be demonstrated that virus tends to die off rather rapidly in storage at 22° C., more slowly but still considerably when frozen; that the yellow areas of mottled leaves are much better sources of virus than the adjacent green areas of the same leaves; that green leaves above the point of inoculation may quickly become strong sources of virus, yet apparently similar green leaves immediately below the point of inoculation remain free of virus for some weeks, later becoming effective sources of virus.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.  
YONKERS, N.Y.

## EFFECT OF PEAT MOSS AND SAND ON ROOTING RESPONSE OF CUTTINGS<sup>1</sup>

A. E. HITCHCOCK

(WITH PLATES V-VII AND FIVE FIGURES)

### Introduction

Studies relating to growth responses in plants have been conducted by numerous investigators over a period of many years. As a result of these investigations, many fundamental facts have been established regarding relationships between the plant and its environment. Similar relationships, however, have not been established for cuttings. For example, it is not known definitely to what extent external factors in a rooting medium may modify the tendency to initiation and growth of roots by cuttings. The fact that sand has been universally employed as a medium in which to root cuttings indicates that it has been generally thought that aeration and aseptic conditions are more important for the production of roots by cuttings than nutrient elements such as are present in soil.

MCCALLUM (14) attempted to prove that environmental influences cannot be regarded as directly causing the adventitious formation of roots or shoots. Light, gravity, and moisture were tested. Portions of the cutting were exposed to partially or completely saturated atmospheres as well as to water. *Phaseolus multiflorus* was mainly used for these experiments, although it was stated that *Salix*, *Helianthus*, *Taraxacum*, and *Tolmiea* responded in essentially the same manner. MCCALLUM concluded that regeneration of roots and shoots is determined by the same internal factors which control the development of dormant buds. He regarded regeneration as inseparable from growth, and much of his work was concerned with the develop-

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

ment of dormant shoot buds. His results do not adequately present or characterize the effect of external conditions on rooting response in a solid medium. So far as polarity is concerned, McCALLUM confirmed the work of VÖCHTING.

VÖCHTING (24) considered polarity of roots and shoots to be due mainly to properties inherent in living matter, although he states that light and moisture may have modifying effects. These conclusions reaffirm the results obtained in earlier experiments. His results show, in addition, that the roots from cuttings in sand were more numerous, larger in diameter, and longer than those from similar cuttings placed in water.

CURTIS (7) reported stimulation of root growth as a result of certain chemical treatments of cuttings prior to their placement in sand. The greatest stimulatory effect on root growth was produced by the use of a 1-2 per cent solution of potassium permanganate. Consistent results were obtained for this treatment in many tests on *Ligustrum ovalifolium*. Increased growth was not obtained, however, when the chemical was added to the rooting medium. Crone's and Knop's nutrient solutions in concentrations of 0.01, 0.10, and 0.50 per cent were found to be injurious to hard wood cuttings of *Ligustrum ovalifolium*.

SMALL (19) tested the effect of adding dilute acetic acid (1:10,000) to coconut fiber and to soil, and reported an increased percentage of rooting, or a reduction in time required for rooting, for all treated lots of cuttings as compared with the untreated ones. This treatment was found to be favorable for many varieties of cuttings. On the other hand, VIERHELLER (23) failed to improve the rooting of apple cuttings when SMALL's acid treatment was used. PHILLIPS (17) likewise obtained no marked improvement in rooting when dilute solutions of acetic acid were added to sand, to soil, or to a synthetic leaf mold, in which cuttings of *Ocotea bullata* were placed. It would appear from these results that all kinds of cuttings will not respond in the same way to a given chemical modification of the rooting medium. PHILLIPS found, however, that when etiolated shoots of *Ocotea* were used for cuttings instead of normal shoots, a more favorable rooting response was obtained. Tests in sand, clay, and synthetic leaf mold gave, in the order named, 30, 20, and 18

per cent rooting as compared with 6 per cent for the best lot of cuttings made from normal shoots. The physiological condition of the material selected for cuttings, therefore, is seen to be of considerable importance when comparing their ability to root in different media.

ZIMMERMAN (25) stated that peat moss was a good medium in which to root *Ilex*, Delaware grape, and some *Viburnum* cuttings. The last usually rooted at the base in sand, whereas in a peat moss medium rooting took place all along the portion of the stem which was buried. It was suggested that peat moss may contain stimulating substances which cause this specific response. The same worker also pointed out that favorable results might be expected with mixtures of peat moss and sand. These mixtures, it was stated, would hold water better than sand, and some benefit might be expected from the presence of peat moss.

SMITH (20) reported on the relation of acidity of the medium and root production in *Coleus*. Although *Coleus* cuttings rooted very readily in coconut fiber (pH 4.5-4.7), it was found that in a series of acid-alkali solutions ranging from pH 4.0 to 9.0, best rooting occurred at pH 7.0. In these solutions there appeared to be a direct relation between pH value and rooting response, if the dry weight of roots per gram of top dry matter was taken as the criterion of growth. SMITH inferred that the favorable rooting in acid coconut fiber was due to the efficient aeration provided by this medium. This conclusion was arrived at from the fact that the bases of cuttings in the fiber gave an immediate fat test with Sudan III, in contrast to a practically negative test for cuttings rooted in solutions. The presence of fatty substances at the base of the cutting was regarded as due to a plentiful supply of oxygen.

A preliminary report has been published on variation in rooting response of cuttings placed in media of different pH values (10). It was pointed out that *Azalea amoena* cuttings rooted well in natural peat moss, but rooted poorly in sand and in neutral peat moss. Cuttings of California privet gave the opposite response in these media. In a mixture composed of equal parts of peat moss and sand, both *Azalea* and privet rooted exceptionally well, indicating that such a mixture might prove favorable for many varieties of cuttings which root poorly in either peat moss or in sand.

KNIGHT and WITT (12) found that for rooting plum cuttings sand was more suitable than mixtures of coconut fiber and loam, loam and sand, or coconut fiber and sand. In these mixtures there was considerable variation in rooting for the four varieties tested, but in only one case was the percentage of rooting as high as that in sand.

The published data show clearly that root production by cuttings of one variety may be very different from that by cuttings of another variety, when both are placed in the same medium. Only a few varieties have been tested by any single worker, however, and comparable data by different workers are available for only a very small number of plant forms. It is evident, then, that before any generalizations can be made regarding the conditions which should obtain in a rooting medium in order to give favorable rooting, many different kinds of plants must be tested. Owing to the need for more specific information relating to this general subject, an experimental study of vegetative propagation was begun in 1925 at the Boyce Thompson Institute for Plant Research in Yonkers, New York. The purpose of this project is to determine, so far as possible, the important factors concerned in propagating plants vegetatively. Many phases of the project are now being studied. This paper reports the findings relating to the effect of peat moss and sand on rooting response of stem cuttings.

An attempt has been made by the writer to cover a wide range of plant types in making these media tests of cuttings. In the experiments reported 91 varieties of plants, including 46 genera, were used. In addition to obtaining data on differences in rooting response for many varieties of cuttings, an attempt has been made to determine to what extent the acid reaction of peat moss is concerned in producing favorable or unfavorable conditions for root production. The results here recorded are based on work which was done over a period of three years.

### Materials and methods

Peat moss, sand, and mixtures of these two constituted the media used in the experiments.<sup>2</sup> Due to the marked contrast in their physi-

<sup>2</sup> Granulated peat moss was obtained from Atkins and Durbrow, New York City. Sand was obtained from the Yonkers Builders Supply Co., Yonkers, N.Y. The sand is reported to come from Cow Bay, Long Island, N.Y.

cal and chemical properties, as shown in table I, peat moss and sand afford unusually good means for obtaining widely divergent conditions without at the same time introducing more than two sets of substratum complexes.

Other phases of the work have shown that the capacity of cuttings to form roots is dependent to some extent upon the age of the wood, season taken, activity of growth, and the amount of leaf surface left on the cutting. These factors were accordingly taken into consideration when selecting material for the experiments. All leafy cuttings were brought into a turgid state, before placement in the rooting medium, by soaking in water for a few minutes or longer if necessary. In the greenhouse, pots were used in most cases to hold

TABLE I  
PROPERTIES OF PEAT MOSS COMPARED WITH THOSE OF BUILDING SAND

PROPERTY	PEAT MOSS	SAND
Reaction (pH value).....	3.6	7.0+
Wt. of water in gm. held by 100 gm. dry weight peat moss.....	1000*	20
Origin.....	Sphagnum bogs	Rock strata
Organic matter content.....	99 per cent	Negligible
Texture.....	Finely fibrous	Coarse crystalline
Weight in gm. of 100 cc. (air-dry).....	15†	162

\* DACHENOWSKI gives a value of 1635.5.

† The moisture content of peat moss in the bale will vary greatly according to the humidity conditions under which it is stored.

the media; outside in the cold frames, cuttings were placed in beds of media protected by an overhead slat shade, no sash being used.

Since sand dries out readily when not covered by sash, an auto-irrigator was used in some of the experiments to provide the rooting medium with a higher and a more nearly constant moisture supply. The auto-irrigator consisted of a small clay pot placed inside of a larger pot, with sand or other media between the walls of the two pots. By keeping a supply of water in the smaller pot, the medium was assured of a continuous and a comparatively constant moisture supply. It was necessary for this purpose to select pots which lost water readily.

All hydrogen-ion measurements were made with a quinhydrone electrode apparatus of a type devised by W. J. YODEN, of the Boyce

Thompson Institute for Plant Research. Samples of the media were prepared by adding a sufficient amount of tap water to produce a few cubic centimeters of free liquid. These were allowed to stand overnight in stoppered flasks. A portion of the sample was then transferred to the electrode chamber by means of a small porcelain spoon. The sample thus measured, in the case of peat moss, was of the consistency of mush. Tap water was used, since preliminary tests showed there was no difference between the values obtained for samples made up with tap water and those made up with distilled water. On duplicate samples of standard buffer solutions the accuracy of the particular quinhydrone apparatus used by the writer was found to be within pH 0.03. When checked with a standard hydrogen apparatus, the agreement was within pH 0.03. A standard calomel half-cell was always used in making the measurements with the quinhydrone apparatus.

The reasons for using a heavy suspension of peat moss rather than an extract are explained in connection with the results showing the buffer capacity of peat moss. A heavy suspension appears to be more representative of the actual conditions in the rooting medium. Although OLSEN and LINDERSTRØM-LANG (16) found a filtrate from soil samples to be less variable than a suspension, they recommend using a minimum of water in making up samples for hydrogen-ion determinations. DOMONTOVITSCH (9) found that many plant juices give the same pH values regardless of whether or not the suspended particles were removed. Tomato leaves, however, gave higher pH values when the centrifuged extract was used than when the original extract was used. In comparing the hydrogen electrode with the quinhydrone electrode for measuring the pH values of plant juices, DOMONTOVITSCH found reasonably good agreement except for turnip and onion, in which cases there were appreciable differences in the readings.

BRAY (3) found that if the particles of a soil suspension were agitated by blowing in hydrogen gas from the bottom of the electrode chamber, the pH value was different from that obtained when the particles were allowed to settle out in the bottom of the vessel. These recent investigations have been particularly concerned with the part which solid materials play in affecting the measurement of



hydrogen-ions by the electrometric method. The tendency appears to be toward the use of suspensions rather than filtrates or extracts.

The time at which a reading should be taken after the quinhydrone is added to the sample will no doubt vary according to the type of material being tested. This applies particularly to suspensions. CRUZ (6) took readings at the end of one minute. SNYDER (21) recommends from one-half to one minute for taking readings of the pH. Neither states why these time periods were chosen. As will be shown by the results obtained for buffer tests, an immediate equilibrium is not reached when quinhydrone is added to a heavy suspension of peat moss.

### Experimental results

A general classification of rooting response for 96 varieties of cuttings is given in table II. Experiments recorded in tables III, IV, and V show the degree of rooting which was characteristic for each of the three groups listed in table III.

Experiment 1 (table III) shows that *Azalea amoena* cuttings rooted readily in natural peat moss and in a mixture of peat moss and sand, but that in neutral sand, neutral peat moss, and in a mixture of sand and neutral peat moss much poorer rooting was obtained. Eleven other experiments with *Azalea amoena* gave essentially the same results. Experiment 2 (table III) is mentioned primarily to show that neutral peat moss which has been previously used has different effects from those of a freshly prepared lot of the same material. Although *Azalea ledifolia* (*indica alba*) does not root so readily or so uniformly as *Azalea amoena*, the relative differences in the amount of roots formed in different media were found to be essentially the same. The difference in the size of the root systems for *Azalea amoena* cuttings rooted in peat moss and those rooted in sand is shown in figs. 1, 2. Uniformity of rooting in peat moss and lack of uniformity in sand are also evident in the same figures.

*Prunus glandulosa* rooted poorly in peat moss, exhibiting an injury similar to that of privet cuttings in the same medium. Excellent root systems were produced in sand, however, and in a mixture of sand and peat moss. These results are recorded in table IV (experiments 5 and 6). The results recorded for experiment 5 are also

TABLE II

CLASSIFICATION OF VARIETIES OF CUTTINGS ACCORDING TO ROOTING RESPONSE IN PEAT MOSS AND SAND

GROUP I CUTTINGS ROOTED READILY IN PEAT MOSS BUT POORLY IN SAND		GROUP II CUTTINGS ROOTED READILY IN SAND BUT POORLY IN PEAT MOSS	GROUP III CUTTINGS ROOTED READILY IN EITHER PEAT MOSS OR IN SAND
Azalea amoena* (6-12) † Azalea ledifolia* (8) Azalea (hardy mollis hybrids) (7) Blue spruce (2-4) Delaware grape (7) Enkianthus campanulatus (9) Ilex crenata (3) Vaccinium corymbosum (6-8)	Asclepias nivea (1-4) Berberis thunbergii (8) Carnation (7) Cofoneaster horizontalis (7) Daphne cneorum (7) Datura stramonium (1) Deutzia gracilis* (6-8) Heliotrope (10) Lagerstroemia indica (8) Ligustrum ibota (var. regelianum)* (8) Ligustrum japonicum (9) Ligustrum ovalifolium* (6, 8, 11) Ligustrum vulgare* (8) Mentha piperita* (7-10) Osmanthus aquifolium (white variegated) (8) Prunus glandulosa (pink variety)* (6-8) Prunus tomentosa* (6) Prunus triloba (12) Rosa hugonis (6) Syringa vulgaris* (6, 7)	Buddleia davidii (8) Buxus sempervirens (7, 11) CalliCARPA purpurea (6-8) Capsicum grossum (4) Coleus blumei (4 var.)* (1-12) Cornus florida (pink) (6, 7) Cornus florida (white) (6, 7) Cornus kousa (6) Cornus mas (6) Dahlia (6 var.) (4, 5, 8-11) Evonymus radicans (7, 8) Evonymus (variegated) (7) Forsythia intermedia (5-9) Forsythia viridissima (5-9) Fuchsia spectosa (3 var.) (6-9) Gardenia florida (2, 7) Geranium (4, 6, 10-12) Hydrangea opuloides (8) Hydrangea petiolaris (6) Ilex aquifolium (8-10) Ilex cornuta (1) Ilex glabra (8-12) Ilex opaca (6, 8-12, 1) Lonicera grafravittissima (7, 8) Lonicera morrowi (7, 8) Lonicera tatarica (7, 8) Philadelphus coronarius (6-8) Philadelphus folconeri (6-8) Philadelphus gordonianus (6-8) Philadelphus grandiflorus (6-8) Philadelphus leuoi (6-8)	Ribes alpinum (6-8) Ribes nigra (6-8) Rosa (American Pillar) (6-9) Rosa (Dorothy Perkins) (6-9) Rosa setigera (5) Rosa (Silver Moon) (7) Salix alba (5, 6, 11) Salvia splendens (5, 8) Sambucus canadensis (6-8) Spiraea (Anthony Waterer) (6-8) Spiraea arguta (6-8) Spiraea nipponica rotundifolia (6-8) Spiraea reevesiana (6-8) Spiraea thunbergii (6-8) Spiraea van houttei (6-8) Symphoricarpos racemosus (6-8) Symphoricarpos vulgaris (6-9) Taxus cuspidata (1, 6, 11) Taxus (weeping variety) (1) Tsuga canadensis (6-8) Ulmus parvifolia (6) Ulmus pumila* (5, 6) Viburnum carlesii (7) Viburnum opulus americanum (6-8) Viburnum opulus sterile (6-8) Viburnum tomentosum plicatum (1-3, 12) Weigela floribunda (6-9) Weigela rosea (7)

\* Cuttings tested in neutral peat moss.

† Numerals in parenthesis indicate months during which cuttings were taken; for example, (6) refers to June.

Note: Cuttings in all three groups rooted readily in a mixture of equal parts of peat moss and sand, with the exception of the five varieties mentioned in the text.

TABLE III  
TYPICAL ROOTING RESPONSE FOR CUTTINGS OF VARIETIES LISTED IN GROUP I

No. OF EXPERIMENT	VARIETY OF CUTTING	DATE	No. OF CUTTINGS IN EACH MEDIUM	RATING OF ROOT SYSTEM IN DIFFERENT MEDIA									
				Sand		Mixture sand and peat moss		Natural peat moss		Neutral peat moss		Mixture sand and neutral peat moss	
				Per- centage rooted	Size root system*	Per- centage rooted	Size root system	Per- centage rooted	Size root system	Per- centage rooted	Size root system	Per- centage rooted	Size root system
1.....	Azalea amoena	7-11 to 8-16-27	40	60	++*	83	+++	90	+++	37	+	50	+
2.....	Azalea amoena	8-28 to 10-18-27	30	60	+++	90	+++	97	+++	76†	+	90†	+++
3.....	Azalea ledifolia	8-28 to 10-18-27	30	22	+++	60	++	56	+++	67†	+++	60†	+++
4.....	Ilex crenata	2-28 to 3-28-27	20	55	+	35	+	72	+++	.....	.....	.....	.....

\* + represents a poor root system; ++ a fair root system; and +++ a normally vigorous root system.

† Medium which had been used previously and was 3 months old.

TABLE IV  
TYPICAL ROOTING RESPONSE FOR CUTTINGS OF VARIETIES LISTED IN GROUP II

No. of EXPERI- MENT	VARIETY OF CUTTING	DATE	No. of CUTTINGS IN EACH MEDIUM	RATING OF ROOT SYSTEM IN DIFFERENT MEDIA									
				Sand		Mixture sand and peat moss		Natural peat moss		Neutral peat moss		Mixture sand and neutral peat moss	
				Per- centage rooted	Size root system	Per- centage rooted	Size root system	Per- centage rooted	Size root system	Per- centage rooted	Size root system	Per- centage rooted	Size root system
5.....	Prunus glandulosa (pink)	6-25 to 8-10-27	15	100	++*	100	++	++	50	++	100	++	++
6.....	Prunus glandulosa (pink)	6-30 to 8-17-27	50	97	+++	78	++	++	24	++	.....	.....	.....
7.....	Deutzia gracilis	6-15 to 8-6-27	20	100	+++	100	++	++	50	++	.....	.....	.....
8.....	Ligustrum ovalifolium	7-1 to 8-11-27	20	70	++	100	++	++	30	+	.....	.....	.....

\* + represents a poor root system; ++ a fair root system; and +++ a normally vigorous root system.

TABLE V  
TYPICAL ROOTING RESPONSE FOR CUTTINGS OF VARIETIES LISTED IN GROUP III

No. of EXPERI- MENT	VARIETY OF CUTTING	DATE	No. of CUTTINGS IN EACH MEDIUM	RATING OF ROOT SYSTEM IN DIFFERENT MEDIA							
				Sand		Mixture sand and peat moss		Natural peat moss		Neutral peat moss	
				Per- centage rooted	Size root system	Per- centage rooted	Size root system	Per- centage rooted	Size root system	Per- centage rooted	Size root system
9.....	<i>Coleus blumei</i>	12-1 to 12-20-26	30	100	+	100	+	100	+	100	+
10.....	<i>Coleus blumei</i>	9-24 to 10-15-27	10	100	+	100	+	100	+	100	+
11.....	<i>Viburnum opulus</i> sterile	6-30 to 8-25-27	20	100	+	100	+	100	+	.....	.....
12.....	<i>Cornus florida</i>	6-16 to 7-26-27	15	35	+	83	+	35	+	.....	.....
13.....	<i>Ilex opaca</i>	9-15 to 11-16-26	15	54	+	85	+	67	+	.....	.....

\* + represents a poor root system; ++ a fair root system; and +++ a normally vigorous root system.

shown in fig. 3. It is important to note that the best rooting of almond cuttings was obtained in neutral peat moss and in the mixture of sand and neutral peat moss.

Five varieties of cuttings listed under group II (table II) rooted poorest in peat moss, only slightly better in a mixture of sand and peat moss, and exceptionally well in sand. These varieties were *Deutzia gracilis*, *Prunus tomentosa*, *Daphne cneorum*, *Ligustrum japonicum*, and *Syringa vulgaris*. All other cuttings in group II rooted fully as well in a mixture of sand and peat moss as they did in sand.

Cuttings listed in group III rooted readily in both peat moss and in sand, although in most cases the best rooting occurred in a

TABLE VI  
VARIATION IN pH VALUE OF CENTRIFUGED PEAT MOSS  
EXTRACT DUE TO DIFFERENT METHODS OF RINSING  
ELECTRODE AND ELECTRODE CHAMBER

RINSED WITH RUNNING TAP WATER (DUPLICATE LOTS)		RINSED WITH TEST SOLUTION	NO RINSING	RINSED WITH DISTILLED WATER
4.36	4.22	3.99	3.94	3.94
4.51	4.49	3.94	3.94	3.94
4.65	4.49	3.94	3.94	3.94
4.41	4.65	3.94	3.94	3.94

mixture of sand and peat moss. Examples of this type are given in table VI. Ten other experiments with *Coleus* showed that in neutral peat moss by far the largest root systems were produced. For *Coleus* cuttings it was immaterial whether peat moss was neutralized by adding calcium carbonate, a calcium carbide waste product, an asbestos product, or by leaching with tap water.

A few experiments were conducted for the purpose of determining the effect of the medium on root growth after the roots had appeared. The results for mint cuttings are given in fig. 4. Similar results were obtained for *Coleus*, except that in this case the cuttings were not so sensitive to peat moss before being transferred. The check lots of cuttings were removed from the medium and replaced at the same time that duplicate lots were transferred from one kind of medium to another. These results show that the rate of root

growth in *Coleus* and mint cuttings is retarded by natural peat moss and accelerated by neutral peat moss.

Results of tests in which an auto-irrigator was used to supply moisture showed that root production could be improved by this

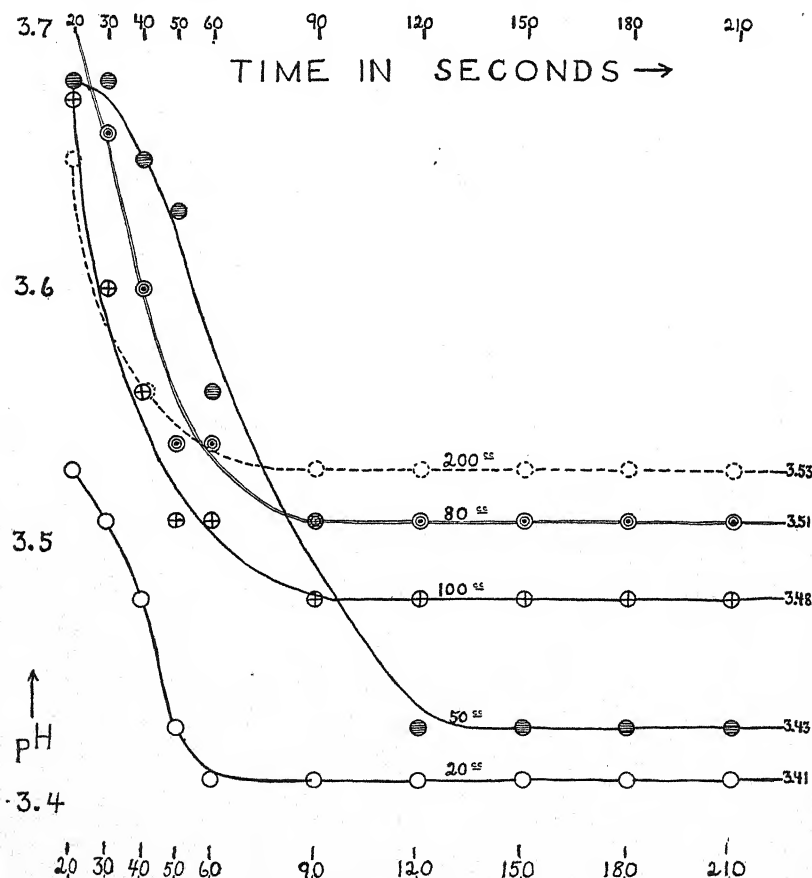


FIG. 7.—Equilibrium relations in electrode chamber for heavy suspension of peat moss, showing change in pH value with time at which readings were taken.

method, especially under conditions of high light intensity. The following cuttings were subjected to the auto-irrigator treatment: privet (3 varieties), *Prunus tomentosa*, Pillar rose, Dorothy Perkins rose, heliotrope, *Deutzia gracilis*, *Spiraea*, *Philadelphus*, *Asclepias*, and *Ilex opaca*. The results for two varieties of privet and for *Prunus tomentosa* are shown in figs. 5 and 6.

## BUFFER PROPERTIES OF PEAT MOSS

Difficulties first met with in measuring the hydrogen-ion concentration of peat moss made it necessary to learn more about the buffer capacity of this material. The data in fig. 7 show the variation in pH value that results when readings are made immediately after quinhydrone is added to a heavy suspension of peat moss in

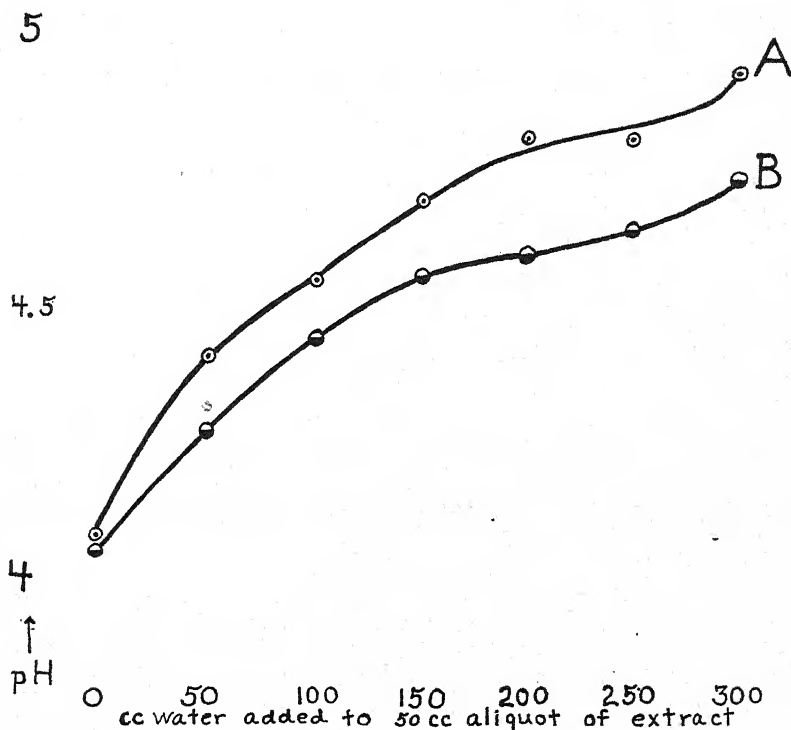


FIG. 8.—Change in pH of peat moss extract due to dilution

the electrode chamber. The change in all cases was in the same direction, that is, toward a more acid value. An equilibrium was practically reached at the end of one minute. Dilution values on each curve represent the amount of water added to 5 gm. of air-dry peat moss. For this particular lot the peat moss contained 50 per cent of moisture, although its moisture content in the bale varied from 30 to 150 per cent, according to the humidity conditions under which it was stored. The 20 cc. dilution curve represents the approximate



minimum moisture content at which a pH measurement of peat moss can be made with the quinhydrone apparatus.

Although the addition of water to peat moss, in great excess of that required to make up a sample for hydrogen-ion determination, does not alter appreciably the pH value of a heavy suspension, the dilution of a peat moss extract causes a marked change in pH value. The results for dilution of an extract are shown in fig. 8. Curves *A* and *B* represent dilution values for extracts from two different lots of peat moss. In both cases the extracts were freed from solid par-

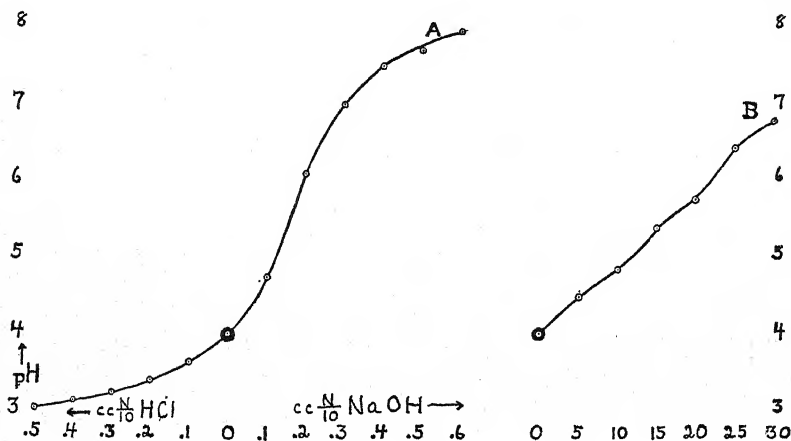


FIG. 9.—*A*, change in pH of peat moss extract due to addition to HCl and NaOH; *B*, change in pH of peat moss due to addition of NaOH.

ticles by centrifuging. These dilution curves show that a water extract is not very efficiently buffered. Further evidence to support this fact is given in fig. 9, which shows the result of adding  $\text{N}/10$  sodium hydroxide and  $\text{N}/10$  hydrochloric acid to 50 cc. portions of peat moss extract. Curve *A* (fig. 9) represents the change in pH value of a peat moss extract due to the addition of acid and alkali. Since only small amounts of either acid or base were required to cause an appreciable change in pH value, the extract cannot be considered as being efficiently buffered. The effectiveness of the solid material in taking out large quantities of base is shown by the results represented in *B*. In this case fifty times the concentration of base was

used, with the difference that the sodium hydroxide was added to the peat moss 24 hours before an extract was made.

In order to determine whether the capacity of peat moss to take up sodium hydroxide is a quantitative reaction, larger aliquots of peat moss and proportionally greater concentrations of base were used. Curves for these results are shown in fig. 10. *A* represents the change in pH value due to the addition of alkali in 5 cc. incre-

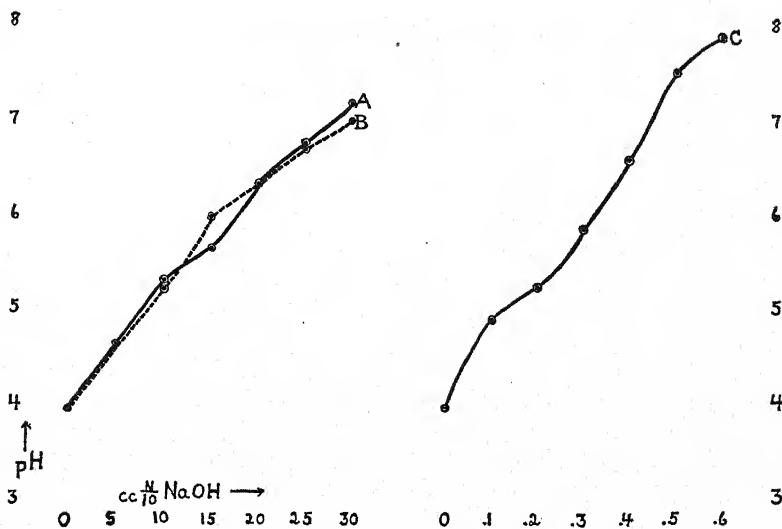


FIG. 10.—*A*, change in pH due to addition of NaOH to 50 cc. portions of peat moss; *B*, change in pH due to addition of four times the amount of NaOH to four times the volume of peat moss as used for obtaining values for *A*; *C*, change in pH of extract from 50 cc. of peat moss due to addition of NaOH.

ments to 50 cc. portions of peat moss. *B* represents the change in pH value due to the addition of alkali in 20 cc. increments to 200 cc. portions of peat moss. In both cases extracts were made 24 hours after alkali was added. *C* represents the change in pH value due to the addition of alkali in 0.10 cc. increments to the extract obtained from 50 cc. of peat moss. Although the same amounts of peat moss were used in obtaining extracts for values in *A* and *C*, the neutralizing capacity (*A*) is shown to be over fifty times that of its extract (*C*).

Since powdered calcium carbonate was usually employed to neutralize peat moss used in media tests, the effect of adding 1 gm. increments to liter portions is shown in fig. 11. *A* and *B* represent the change in pH value for an extract and a heavy suspension respectively, obtained from peat moss to which had been added 1

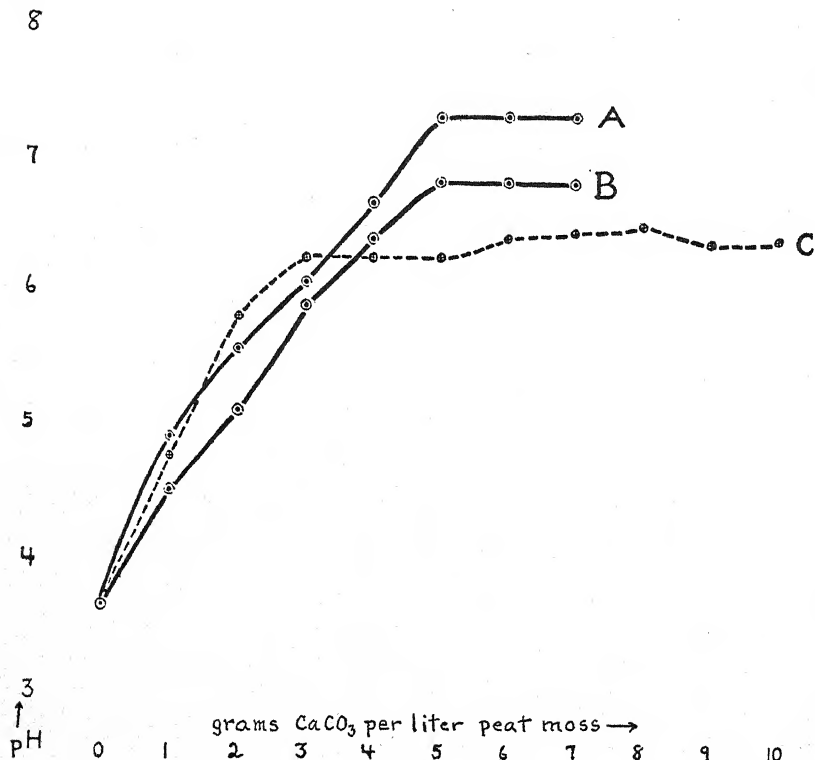


FIG. 11.—Change in pH of peat moss due to addition of increasing amounts of  $\text{CaCO}_3$ .

gm. increments of carbonate. Readings for these two curves were taken 5 days after addition of the carbonate. The results after 2 days for another lot of peat moss are shown in *C*. Higher pH values were invariably obtained for extracts than were obtained for heavy suspensions. This fact held true for both the natural peat moss and for that to which carbonate had been added. The time for complete neutralization with an excess of carbonate depends upon the method

of mixing in the carbonate. Ten grams of carbonate per liter of moistened peat moss will bring about neutralization in 24-48 hours if the material is thoroughly mixed and repeatedly squeezed with the hands. Sufficient water must be added to give the consistency of mush.

When the quinhydrone electrode and the electrode chambers were rinsed with running tap water, an error of considerable proportions was introduced. Rinsing with distilled water or with the test solution caused no error. Likewise, no error was introduced if the solution was completely thrown out of the electrode chamber without any subsequent rinsing procedure. Comparisons of different methods of rinsing are given in table VI.

#### Discussion of results

Contrary to the general belief that sand is the most suitable medium in which to root most kinds of cuttings, it was found that this held true under the conditions given for only 6 out of 96 varieties tested (table II). On the other hand, a mixture of peat moss and sand proved to be far superior to sand, inasmuch as 90 out of 96 varieties of cuttings rooted readily in this mixture. These results are of particular interest, since the advice in textbooks on propagation and in a recent paper by STEWART (22) is to the effect that "clean sharp sand" be used for rooting most kinds of cuttings.

The classification of cuttings according to their rooting response, as given in table I, applies in some cases only to cuttings taken within a limited period during the year, although most of the cuttings were taken from June 1 to October 1. *Prunus tomentosa* rooted readily when taken from May 15 to June 20, but much poorer rooting occurred after this period. *Cornus florida* cuttings taken in June rooted more readily than those taken at any other time. Maximum rooting for blue spruce occurred from February 15 to April 15. In contrast to the varieties just mentioned, *Azalea amoena* and California privet cuttings rooted at all times of the year. It is readily seen, then, that seasonal variation is of considerable importance in testing the effect of the medium on root production by cuttings.

In most cases species of the same genus responded similarly in a given medium. *Ilex crenata* and *Rosa hugonis* were exceptions to

this rule. Whereas the former rooted best in peat moss and the latter rooted best in sand, four other species of both *Ilex* and *Rosa* rooted best in a mixture of sand and peat moss. *Ilex opaca* taken in the fall appeared to root more readily in peat moss than when taken during December and January. *Daphne cneorum* was particularly sensitive to peat moss when young shoots were used, but with more mature shoots this effect was much less noticeable. Although blue spruce cuttings taken in late winter and early spring rooted best in peat moss, young shoots of the active spring growth were injured in the same medium. Such seasonal variations in rooting make a rigid classification of cuttings practically impossible, unless it be confined to a definite season of the year. Inasmuch as all of the varieties listed in table II were not tried at all times of the year, a complete seasonal classification could not be made.

For those cuttings which rooted best in peat moss, or in a mixture of peat moss and sand, the appearance of roots occurred at an earlier period, and the rate of growth was more rapid than for similar cuttings placed in sand. This relation held true for cuttings of woody plants but not for *Coleus* and mint, in which cases only a difference in rate of root growth was evident. Although in some cases, as for example with *Azalea amoena*, a fairly high percentage of rooting was obtained in the unfavorable medium, there were very marked differences in the size of the root systems, as illustrated in fig. 1.

Uniformity of root production in peat moss, in contrast to lack of uniformity in sand, is an important feature of the results shown in figs. 1, 2. Since conditions favoring rapid evaporation obtained in the greenhouse during the course of the experiment (August 28 to October 18, 1927), lack of uniformity in sand may be accounted for principally by variation in moisture conditions in different lots of the same medium. Variation in rate of water loss from different pots, with possibly unequal amounts of water added, was no doubt the cause of different moisture conditions in similar lots of sand. Peat moss, being such an efficient moisture retainer, does not dry out readily, however, and hence does not reach the critical low moisture content at which drying of cuttings takes place.

In connection with the subject of moisture content of the medium, it is of interest to note the results of tests by KNIGHT and WITT

(12). For fruit tree cuttings placed in soil under outside conditions, these investigators found that the best rooting was correlated with the least amount of rainfall, and that poorest rooting was correlated with the greatest amount of rainfall. These results are contrary to those obtained by the writer when using peat moss, sand, and a mixture of these two media. No doubt the general complexes of conditions are quite different in the two cases and different varieties of cuttings were used, so that a generalization based upon one set of conditions may not hold true for another. In contrast to the results obtained by KNIGHT and WITT for cuttings in soil, the writer found that a high moisture content, furnished by an auto-irrigator, favored root formation in many varieties of cuttings. Soft, succulent cuttings were usually rooted more readily than were more mature cuttings of the same plants. While this method was especially effective for sand, rooting in peat moss was also improved by auto-irrigation, more particularly when excessive evaporation conditions were provided such as are given by high light intensity, low humidity, and a relatively high temperature.

Cuttings which rooted poorly in peat moss usually showed definite signs of injury. Browning of the basal cut surface and sometimes the lower one-fourth of the cutting often occurred. This injury was particularly noticeable in the case of *Ligustrum*, *Syringa*, and *Prunus*. No callus was formed when this type of injury occurred. Partial or complete neutralization of peat moss allowed formation of good callus, and prevented the injury just described. It was necessary to decrease the acidity only slightly, that is, from pH 3.6 to 4.1, in order to prevent this type of injury. Hard wood cuttings of *Ligustrum ovalifolium* and *Syringa vulgaris* taken in the winter did not show this injury, and good callus formation was obtained on these cuttings even in peat moss of a high moisture content. It seems of interest to point out that in peat moss of a low moisture content (140 per cent) good callus formation occurs on many hard wood cuttings. At this low moisture content peat moss feels dry to the touch, yet a reading with a wet-and-dry bulb apparatus indicated a relative humidity of 96 per cent. The wet-and-dry bulb apparatus was buried in the peat moss, and the reading was made after a temperature equilibrium had been reached. Since

cuttings were rooted regularly in peat moss containing 500-800 per cent of moisture, it is readily seen that this medium furnished an unusually peculiar set of moisture conditions.

That the acidity of peat moss cannot be considered as the single controlling factor determining its value as a rooting medium is shown by fig. 3. Inasmuch as similar results were obtained with other cuttings listed in group II (table II), these results appear to be particularly significant. If the pH value of the medium were considered to be the limiting factor, there should be comparable root systems in lots A, D, and E, with lot B intermediate between lot C and lot A. Such responses did not occur. On the other hand, if mechanical texture such as provided by a mixture of sand and peat moss (lot B) were the limiting factor, then neutralization of the mixture should not have given the noticeable improvement shown in lot E.

It is difficult to explain why a difference of pH 0.6, the approximate difference in pH value between peat moss and a mixture of peat moss and sand, should allow such marked difference in rooting as was regularly shown. If it is assumed that the pH value of the mixture (4.1-4.4) is that at which maximum root formation occurs for cuttings listed in group II (table II), then a more favorable response should not occur in neutral media as is shown in lots D and E (figs. 3, 4). Such a complicated situation can be explained only on the basis that a combination of factors is operating to bring about a given type of rooting response. It is evident, however, that the pH value of peat moss is in some cases one of the most important factors, especially in connection with a direct injury to the cuttings, but in some cases affecting the rate of root growth. For cuttings which are not injured, however, the most important factor in peat moss appears to be the substances it contains (other than its acid reaction) which promote and maintain a rapid rate of root growth.

In addition to experiments with cuttings, the buffer tests on peat moss show that this material furnished conditions that are extremely difficult to analyze. Inasmuch as the hydrogen-ion concentration of peat moss varies according to the method of preparing samples for measurement, it is quite likely that the pH value as measured by the quinhydrone electrode, or by other means, does



not represent the actual hydrogen-ion concentration with which the cutting or roots have to contend. Regardless of the errors which may be involved when pH measurements are made on heavy suspensions of peat moss, it would appear that this type of sample is a more representative one than is an extract of the same material. The capacity of peat moss to take up such large quantities of sodium hydroxide, as shown by the titration curves, indicates that a solid medium such as peat moss must furnish a far more complex set of equilibrium relations than does a liquid medium which contains similar solutes.

Some varieties of cuttings rooted best when the reaction of the medium was comparable with that in which the parent plants are known to produce maximum growth. This is particularly true for *Azalea*, blueberry, lilac, and privet. While it is not the purpose of this report to follow out such comparisons, the results obtained indicate that cuttings of many plants will respond in a similar manner.

Peat moss and a mixture of peat moss and sand will leach sufficiently for a change of approximately pH 0.5 to occur during the course of an experiment, that is, in from six to ten weeks. Whereas the initial pH value of peat moss is 3.6, its final pH value at the end of an experiment would be 3.9-4.1. When the same peat moss was used again for privet cuttings, no marked injury resulted of the kind typical for freshly prepared peat moss. This fact further substantiates the idea that the critical pH value at which cuttings of privet are injured lies more specifically between 3.6 and 4.1. Although *Azalea* cuttings rooted readily in this type of "used" peat moss, root growth on privet cuttings was not so good as that obtained in a mixture, or in neutral peat moss. As was pointed out in experiments 2 and 3 (table III), *Azalea* cuttings rooted much better in a mixture of neutral peat moss and sand which had been previously used, than in a freshly prepared mixture. *Rhododendron* cuttings (*R. maximum*) formed excellent root systems in a leached peat moss which had previously been used in other experiments. The reaction of this medium four months after the original leaching, and at the time the *Rhododendron* cuttings were well rooted, was pH 6.76, showing practically no tendency toward reversion to the acid form. The results with *Rhododendron* are of particular interest,

since cuttings of this plant would be expected to respond the same as *Azalea*. COVILLE (4, 5) has shown that *Rhododendron* seedlings will grow well only in an acid medium, regardless of the amount or type of humus material present. It is possible, of course, that even though *Rhododendron* cuttings were readily rooted in a leached peat moss, they would not continue to grow for an unlimited period. Furthermore, even though root growth were favorable in leached peat moss, shoot growth might not be correspondingly good over a period of several years.

Neutralization of peat moss by alkaline reagents or by leaching with tap water causes the material to change from a light brown to a very dark brown, almost black. This change takes place within a day or two after the reagents have been added, and darkening continues as time goes on. If excessive amounts of alkali are added, the change in color is immediately produced. Change in color no doubt accompanies a chemical reaction. According to ITANO's work (11), it seems quite possible that adjustment of the peat moss to a neutral reaction will allow of active bacterial decomposing action. The change just described may have some bearing on the difference in rooting obtained in used neutral peat moss.

*Ligustrum japonicum* rooted exceptionally well in sand. No roots were produced in peat moss during the time of the experiment. When the cuttings were transferred from peat moss to sand, however, roots appeared in two weeks' time. Such a result brings up the question as to whether the failure of root protrusion was due to lack of root initiation, or whether the root initials formed but failed to grow out through the bark. Although no anatomical studies were made on this material, it is believed that this phase of the general problem of vegetative propagation is extremely important. It is not definitely known whether for some kinds of cuttings different conditions in the rooting medium are required for root initiation from those required for root growth. Experiments with *Coleus* and mint cuttings indicated that for these particular plants differences in rooting in different media were due solely to factors which influenced the rate of root growth.

The efficiency of a mixture of peat moss and sand appears to be due mainly to its relatively high moisture-retaining capacity, to the

presence of growth-promoting materials furnished by the peat moss, to efficient aeration, and in some cases (*Azalea*, for example) to its acid reaction. The part which sand plays in this mixture (aside from that of dilution) is not clear. Both peat moss and sand are well aerated, so that efficient aeration in the mixture cannot be due to sand alone.

It appears from the results with cuttings that root growth may not be confined to such narrow ranges of pH values as has been supposed. Complex relations furnished by solid materials and many kinds of ions in rooting media must be considered as having a pronounced modifying influence on the extent to which the hydrogen-ion will exert its particular influence on root growth. SMITH's work on *Coleus* has an interesting bearing on this phase of the subject.

SMITH (20) found that root formation in *Coleus* cuttings attains a maximum in liquid media at pH 7.0, whereas in coconut fiber excellent rooting was obtained at a much more acid value (pH 4.5-4.7). This fact appears to be of particular importance, since the pH value of coconut fiber is comparable with that of a mixture of sand and peat moss. The writer found that in acid peat moss (pH 3.6) and in neutral sand *Coleus* cuttings rooted equally well. In the mixture of sand and peat moss (pH 4.1-4.4) root growth was invariably better than in either peat moss or in sand. The pH value of this mixture, as well as that of coconut fiber, was found by SMITH to be the acid limit (that is, pH 4.5) for *Coleus* cuttings placed in liquid media.

In view of these results with *Coleus*, it does not appear that data obtained for cuttings rooted in liquid media can always be used to explain the limiting factors for root production in solid media. SMITH's statement that, other conditions being equal, a neutral reaction of the medium was best suited for root production in *Coleus* cuttings, cannot be regarded as a very safe guide, for it is these "other conditions" which are often of the greatest importance. Differences in time of root protrusion at different pH values found by SMITH were not noted by the writer for *Coleus* cuttings placed in peat moss and sand. Observations by the writer included an examination of cuttings in pots of media and in glass tubes. In the latter case, cuttings of *Coleus* were placed next to the glass and fully

exposed to view. Roots appeared from cuttings in peat moss, in neutral peat moss, and in sand at the same time, at least during the same day, yet very marked differences in rate of root growth were observed thereafter in the different media.

Whether peat moss was furnished in its natural acid state, neutralized, or mixed with sand, a more rapid rate of root growth occurred in a medium containing peat moss than in one containing only sand. This brings up the question of the nutrient conditions which are furnished by peat moss. REID (18) pointed out that for tomato cuttings a low nitrogen value in the medium was more favorable for root growth than a high one. Such a result, however, was dependent upon the availability of carbohydrates in the cutting. Whether this relation holds true for other kinds of cuttings has not as yet been demonstrated. In order to gain some idea of the nutrient value of peat moss, tomato and buckwheat seedlings were grown in soil, natural peat moss, neutral peat moss, acid sand, and in neutral sand. Both of these species were able to grow to maturity in peat moss. The growth of tomato in natural peat moss was noticeably retarded during the first few weeks, but later the plant continued in what appeared to be a normal rate of growth, and set fruit. The tomato placed in neutral peat moss was at all times in a more vigorous state of growth than any of the others, including that in soil. Buckwheat did not show such marked variation in the different media, all plants setting fruit and attaining about the same vegetative growth. While this particular set of experiments cannot furnish any quantitative information, it shows that the nutrient value of peat moss is sufficiently high to be an important factor in determining the type of response in this medium.

BOTTOMLEY's experiments (1, 2) with "bacterized peat" showed that the products of bacterial action of peat would stimulate growth of certain plants, especially that of *Lemna minor*. Raw, unbacterized peat would not do this. In connection with this work it is interesting to note the results which ITANO obtained with a Michigan acid peat. The normal alkaline permanganate soluble nitrogen was increased from 5.9 to 71.5 after adjusting the medium to pH 7.0 and adding accessory food materials containing vitamin B. Whereas BOTTOMLEY obtained his "growth-promoting substances" (auximones) from bacterial action on peat, ITANO added "growth-pro-

moting substances" to peat in order to promote bacterial decomposition.

The variation in chemical and physical properties of different types of peat, as shown by DACHNOWSKI (8), makes a comparison of growth responses in various types of peat or peat moss media a somewhat doubtful procedure. No doubt the failure of some workers to repeat BOTTOMLEY's experiments has been due to the fact that the type of peat was different from that used by BOTTOMLEY. DACHNOWSKI makes a definite distinction between "peat" and "peat moss." The type used by the writer is designated "granulated peat moss." This is a standard commercial product which comes from bogs in Germany. It corresponds to the type which DACHNOWSKI classifies as a poorly disintegrated bog moss type (Section C, No. 7, p. 19). Over a period of three years this peat moss has proved to be of uniform texture and of the same acid reaction.

While the use of peat moss as a rooting medium for cuttings is by no means new, its use previous to 1925 for such a purpose in this country was noticeably limited. During the last two years, however, peat moss has been used rather extensively, not only as a medium in which to root cuttings, but also as a means of improving soil conditions. According to frequent reports in *Gartenwelt*, peat moss (torfmull) has been in more general use in Germany than in this country, but no attempt has been made to furnish detailed experimental data on comparative rooting responses of cuttings in peat moss and in sand.

### Summary

1. According to their rooting response in peat moss and in sand, 96 varieties of cuttings (including 46 genera) have been classified into three groups. Cuttings which rooted readily in peat moss but poorly in sand are placed in group I; those which rooted readily in sand but poorly in peat moss are placed in group II; cuttings which rooted readily in either peat moss or in sand are placed in group III.
2. The fact that cuttings in all three groups rooted readily in a mixture composed of equal proportions of peat moss and sand (with the exception of five varieties in group II) indicates that this mixture is superior to sand as a general medium in which to root cuttings. Although the pH value of the medium was an important factor in determining the type of rooting response of some varieties

of cuttings, it was not the single limiting factor. The critical acid value, at which injury to the cuttings listed in group II occurred, was found to lie between pH 3.6 and 4.1. For the same varieties of cuttings callus formation was inhibited at pH values more acid than pH 4.1.

3. Whether peat moss was furnished in its natural acid state, neutralized, or mixed with sand, a more rapid rate of root growth occurred in a medium containing peat moss than in one containing only sand. Good rooting occurred for most varieties of cuttings over an acid range of pH 4.5-7.0.

4. Uniformity of rooting response of *Azalea amoena* cuttings in peat moss is attributed to the efficient moisture-retaining capacity of this medium. An increased moisture content of sand, as furnished by auto-irrigation, showed that in many cases, but especially under conditions of high light intensity, a more favorable rooting response was obtained.

5. For *Coleus* cuttings the conditions in the medium influenced the rate of root growth rather than the time of root protrusion. Cuttings of *Ligustrum japonicum* which failed to root during two months in peat moss, rooted in two weeks when transferred to sand, indicating that root initiation had probably taken place, but that unfavorable conditions provided by peat moss prevented root protrusion.

6. The efficient buffer capacity of peat moss was found to be due principally to the solid material, and not to the solutes in an extract. Methods for preparing samples and for making pH determinations are described in detail. Extracts of peat moss were found to give higher pH values than heavy suspensions.

Experiments here reported were carried out in the laboratories of the Boyce Thompson Institute for Plant Research. The writer is greatly indebted to members of the staff of the Institute and to Professors R. A. HARPER and S. F. TRELEASE of Columbia University for helpful suggestions and criticisms given during the progress of the work.

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## EXPLANATION OF PLATES V-VII

### PLATE V

FIG. 1.—*Azalea amoena* cuttings rooted in different media: A, peat moss medium (pH 3.6); B, bank sand medium (pH 5.8-6.2); C, Bay sand (Long Island, N. Y.) medium (pH 7.0+).

FIG. 2.—*Ligustrum vulgare* cuttings rooted in different media: A, peat moss medium (pH 3.6); B, bank sand medium (pH 5.8-6.2); C, Bay sand (Long Island, N. Y.) medium (pH 7.0+).

### PLATE VI

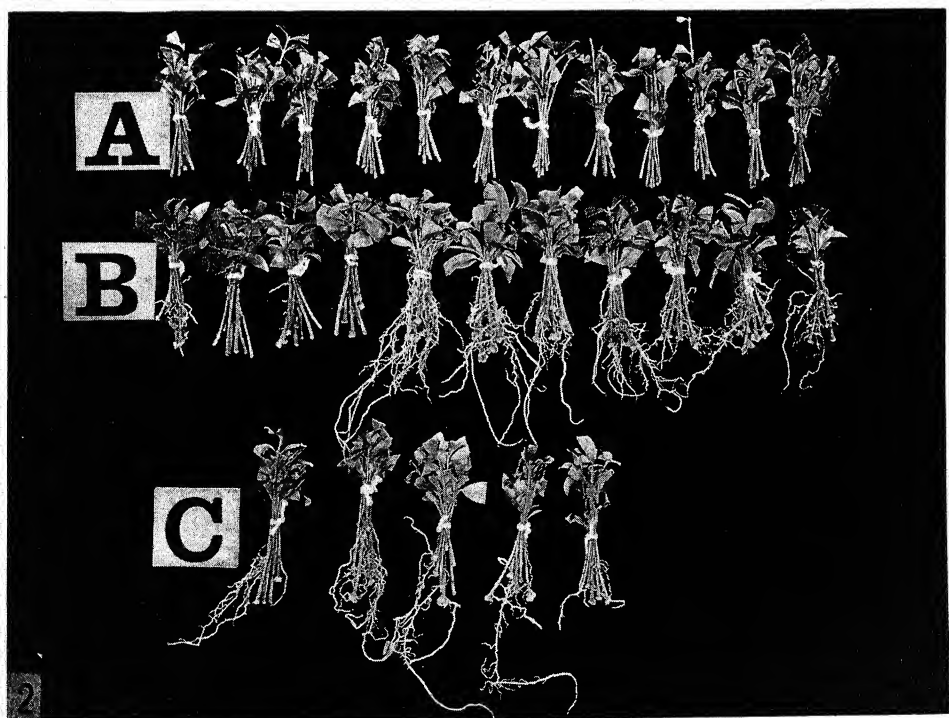
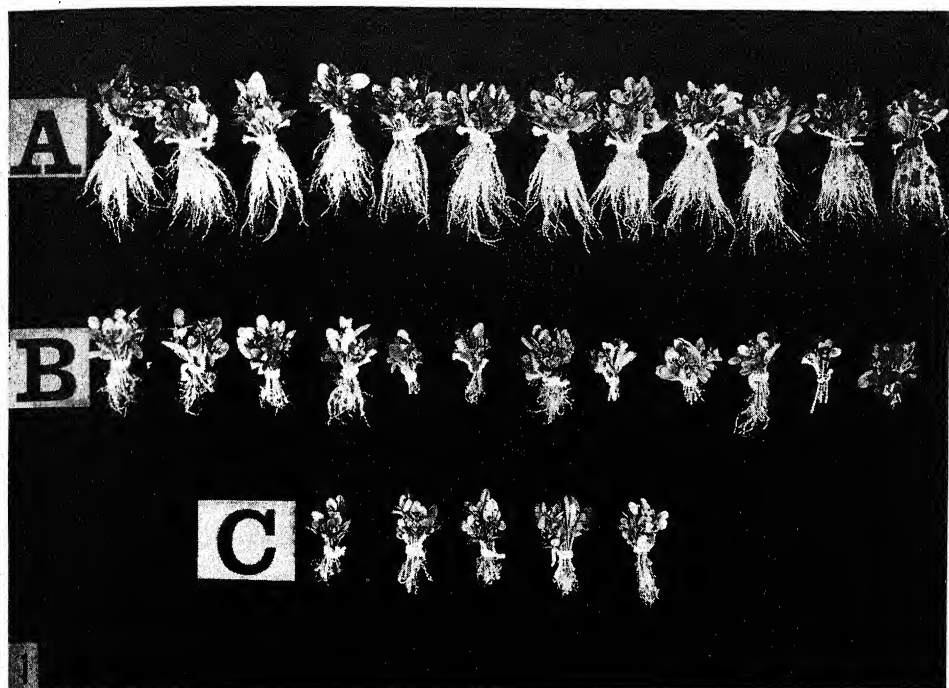
FIG. 3.—Cuttings of *Prunus glandulosa* rooted in different media: A, sand medium (pH 7.0+); B, mixture of peat moss and sand (pH 4.1-4.4); C, peat moss medium (pH 3.6); D, neutral peat moss medium (pH 7.0-7.3); E, mixture neutral peat moss and sand (pH 7.0-7.2).

FIG. 4.—Cuttings of *Mentha piperita* rooted in different media: A, sand medium (pH 7.0+); B, peat moss medium (pH 3.6); C, neutral peat moss medium (pH 7.0-7.3); D, neutral peat moss (pH 7.0-7.3) for ten days, then transferred to peat moss (pH 3.6); E, peat moss (pH 3.6) for ten days, then transferred to neutral peat moss (pH 7.0-7.3).

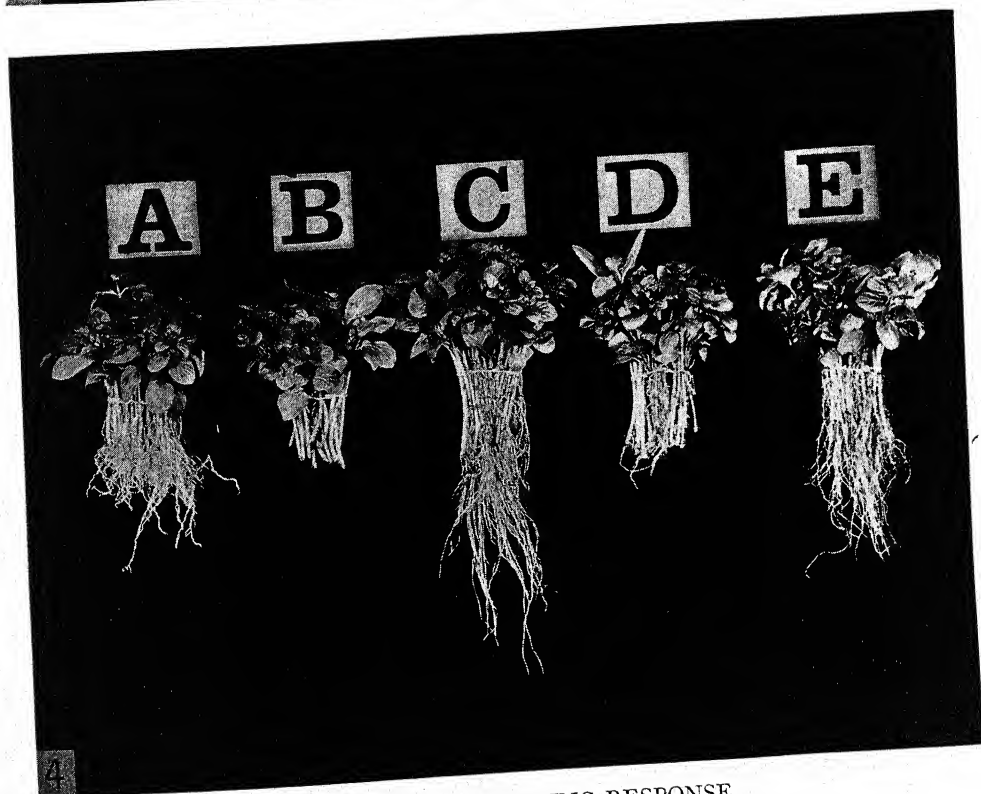
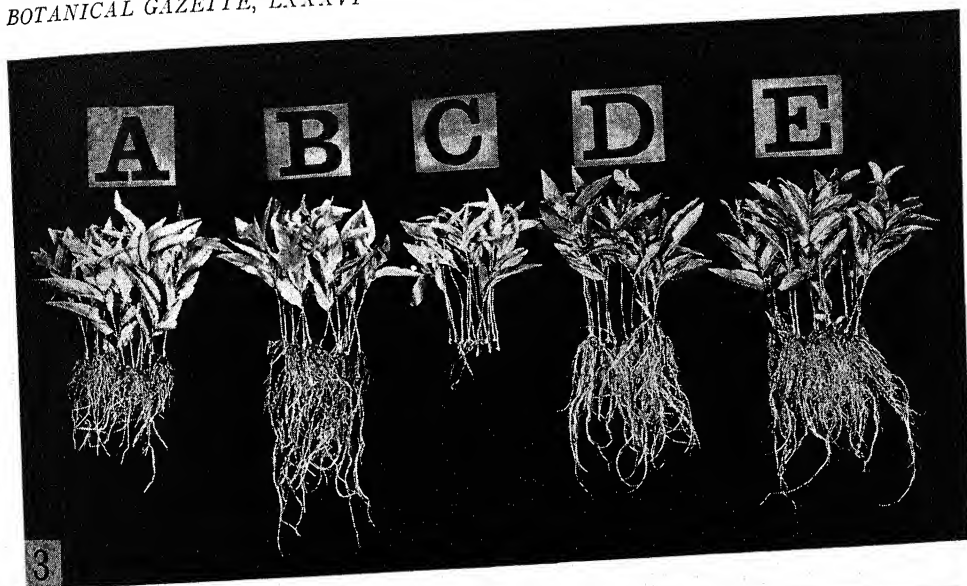
### PLATE VII

FIG. 5.—Cuttings of *Ligustrum vulgare* (top row) and *Ligustrum ibota* var. *regelianum* (bottom row) rooted in different media; effect of auto-irrigation also shown: A, peat moss medium not irrigated; B, peat moss medium auto-irrigated; C, neutral peat moss medium not irrigated; D, neutral peat moss medium auto-irrigated; E, sand medium not irrigated; F, sand medium auto-irrigated; G, sand medium not irrigated (this particular lot of cuttings was shaded daily until 1:00 P.M.).

FIG. 6.—Cuttings of *Prunus tomentosa* rooted in sand: A, auto-irrigated; B, check (not irrigated).

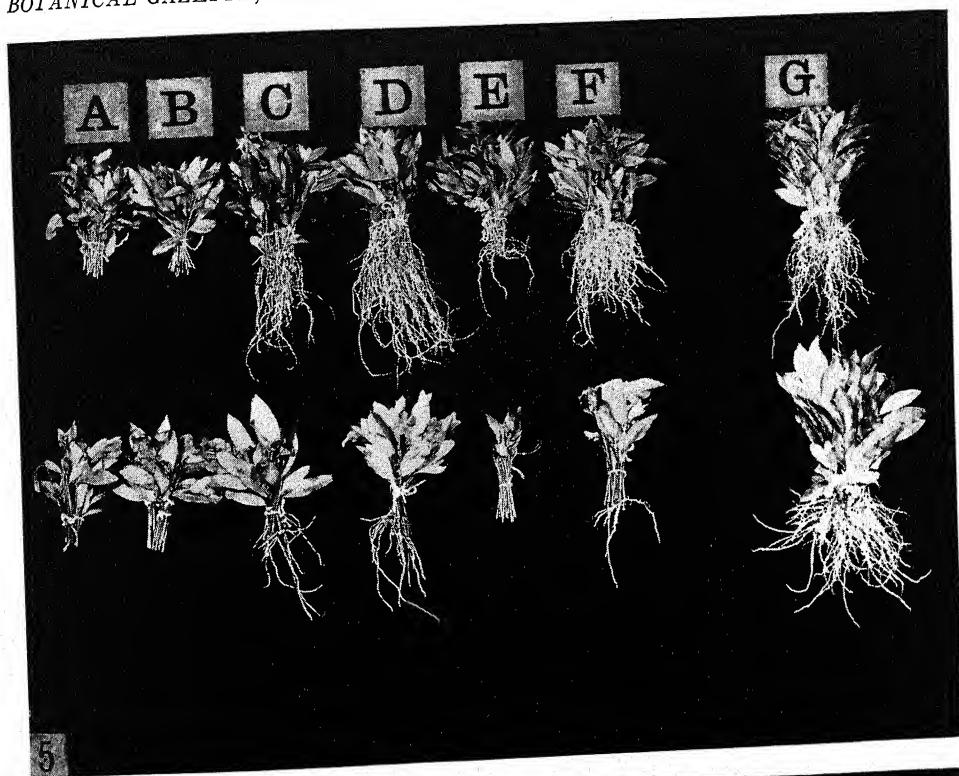


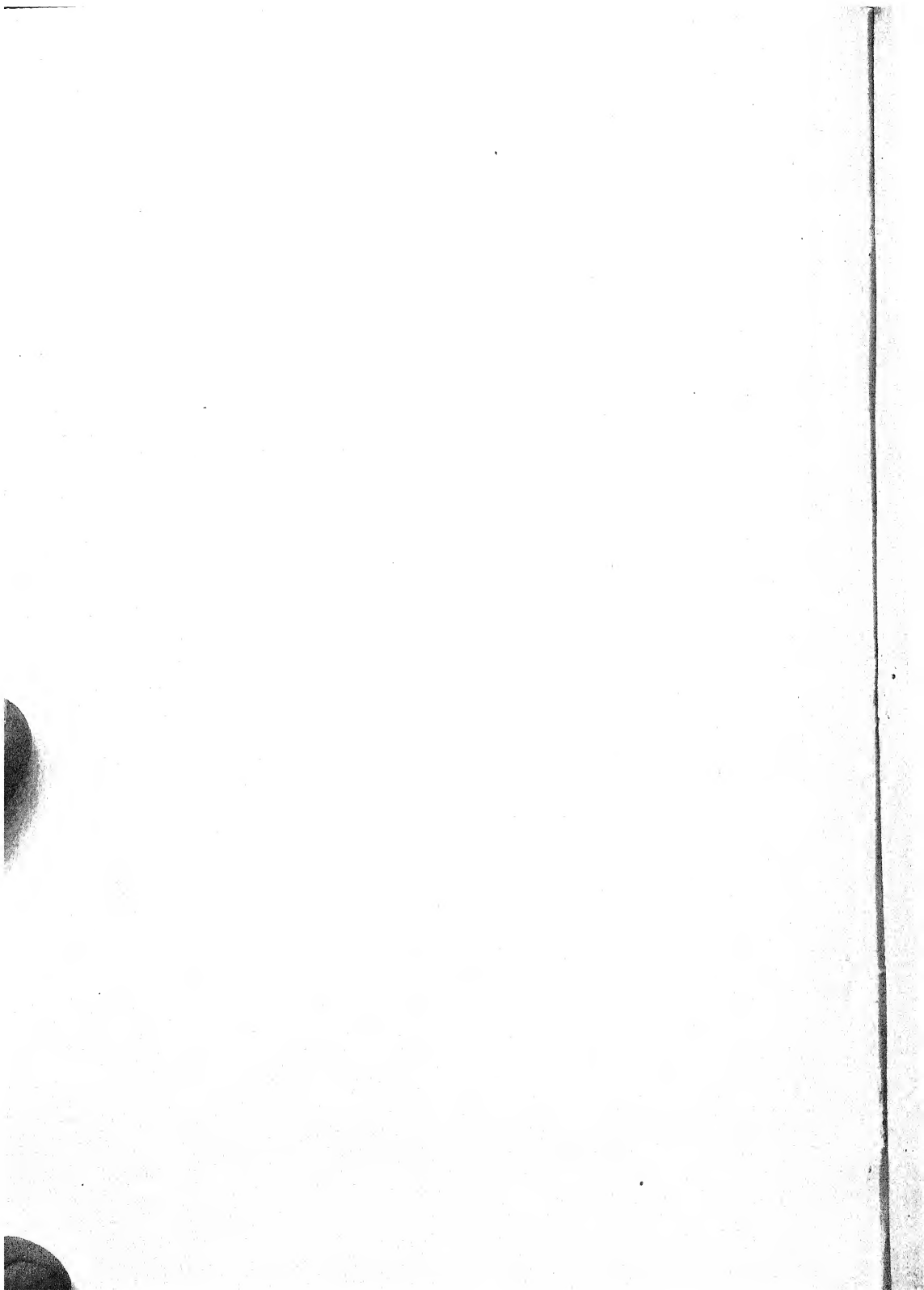




HITCHCOCK on ROOTING RESPONSE









## ROOT FORMATION AND FLOWERING OF DAHLIA CUTTINGS WHEN SUBJECTED TO DIFFERENT DAY LENGTHS<sup>1</sup>

P. W. ZIMMERMAN AND A. E. HITCHCOCK

(WITH SIX FIGURES)

### Introduction

Dahlias propagated from cuttings during late summer and fall vary in response as the days become shorter. Cuttings taken in August produce normal fibrous roots and become plants of good size before flowering. Cuttings taken in late September and October produce storage roots at the sacrifice of fibrous roots, and flower when only a few inches high. Often no roots at all are formed on cuttings taken in October, but the basal ends of the stems serve as storage centers. Occasionally the buds along the stem become storage organs, swelling and resembling tubers. When six hours of extra light are used to supplement the daylight, the plants produce normal fibrous roots and are slow to flower. GARNER and ALLARD (5), ARTHUR and GUTHRIE (1), and ARTHUR (2) have reported many plant species which flower more readily in short day lengths than in long days. GARNER and ALLARD reported one variety of dahlia, John Ehlich, which flowered on July 8 in the 10-hour day as compared with September 27 for the check grown in the natural day length. They also reported that storage roots and tuber formation of several species of plants are affected by day length. Two unnamed dahlia varieties did not show the usual "tuber" formation when grown in lengthened illumination periods. A bean (*Phaseolus multiflora* Willd.) and a yam (*Dioscorea alata*) formed larger storage roots in 10- to 12-hour days than in the full day length of summer. McCormick potatoes did not tuberize with extra illumination added to the usual day length of summer. Artichokes (*Helianthus tubero-*

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

*sus*) in short days formed mature looking tubers in contrast with the slender, immature types of the long day plants.

The purpose of this paper is to report the response of cuttings from several varieties of dahlia when subjected to different day lengths.

### Methods and materials

During the late summer and fall of 1926, cuttings were taken at one-week intervals from dahlias growing on the Institute grounds and were planted in the greenhouse in sand or mixtures of peat moss and sand. The material was generally young shoots coming from the main stem rather than from the ends of large active branches. In this way it was possible to get material free from flower buds. After cuttings were rooted, they were potted in good garden soil and grown either in a greenhouse receiving normal fall light or in an adjoining greenhouse where they were given extra light. The extra light was given from sunset to 12 o'clock midnight by a 1500-watt nitrogen bulb.

Seedlings which were also included in the experiment were from a promiscuous collection of seeds planted on November 3. About 1000 seedlings resulted, and from these the best plants were selected for experimental work.

During 1928 the plants used came, for the most part, from select material purchased from Downs Dahlia Farm, Clayton, New Jersey, and from Fisher and Masson, Trenton, New Jersey. The storage roots were placed in a peat moss medium, and cuttings were made as the shoots appeared above the surface. These cuttings were placed directly into pots containing a mixture of peat moss and garden loam. Later the established plants were repotted in fertilized soil and subjected to different amounts of light. One lot was grown outdoors as a check; a second lot by the check was given 7 hours of daylight and the remainder of the day in the dark room; a third lot by the check was given 9 hours of daylight and the remainder of the day in the dark room.

Nitrate tests were made with a solution of diphenylamin in sulphuric acid (0.1 gm. of diphenylamin to 10 cc. of 75 per cent  $H_2SO_4$ ).

Hydrogen-ion measurements were made with a quinhydrone ap-

paratus. Stems and leaves were cut into small pieces and then crushed in a mortar. Some of the crushed plant material, as well as the juice, was transferred to the electrode chamber. It will be noted that the sample thus measured contained both tissue and its expressed juice.



FIG. 1.—*Dahlia variabilis* (Jersey's Beauty) from cuttings, August 26 to November 15

### Results and discussion

EXPERIMENTS WITH FALL CUTTINGS.—Cuttings taken on August 26 from dahlia varieties, such as Jersey's Beauty, Mrs. I. de ver Warner, or Peg-O-My-Heart, formed normal fibrous roots by September 17 and many flowered by November 15. They did not flower uniformly, but continued up to December 15. At the time of flowering the plants varied from 1 to 3 feet in height (fig. 1). Five plants

given 6 hours of extra light had not flowered by February 7, and neither had they formed storage organs. Fibrous roots and large tops developed at the expense of storage roots. The extra light plants



FIG. 2.—*Dahlia variabilis* (Jersey's Beauty) cuttings, October to December, as they appeared when taken from rooting medium; note almost complete absence of fibrous roots; upper figures, main roots developed are storage organs; lower figures, left, storage in base of new shoot arising after cuttings were made; middle, basal buds form storage organs comparable with tubers; right, portions of cutting, as stem and leaf petiole, serve as storage organs.

ranged from 3 to 4 feet in height, while checks varied from 1 to 3 feet in height.

Cuttings taken from the same varieties on September 26 produced a few fibrous roots, but there was a tendency to produce storage roots while still in the rooting medium. Flower buds were formed when the plants were 10-12 inches high. Flowering and the formation of storage roots took place concurrently from October to December.

Cuttings taken from October 15 to October 28 developed storage roots, as a rule, instead of the normal fibrous roots characteristic for early cuttings. In many instances no roots formed, but various parts of the stems became storage organs (fig. 2). In some cases buds developed into organs resembling bulbs or tubers; in others the stems became storage centers; and in still others remaining portions of leaf petioles were enlarged. Where roots formed, as a rule they were few and mostly of the storage type. A few cuttings without roots flowered while in the rooting medium.

EXPERIMENTS WITH SEEDLINGS.—Approximately 100 seedlings grown from seeds planted November 3 were treated with normal and extra light. Much variation was evident, but the prevailing tendency was for the plants to flower and form storage roots in the short winter days. Plants given extra light formed fibrous roots only, and did not flower during the course of the experiment (fig. 3). Seedlings first developed a fibrous root system from the hypocotyl, and when the plants were 4 or 5 inches high, special roots appeared just below the cotyledons. These roots had a fleshy appearance and quickly developed into storage organs during the short days.

EXPERIMENTS WITH SPRING AND SUMMER CUTTINGS.—On June 8, thirty Jersey's Beauty dahlia plants, half from cuttings and half with tubers attached, were divided into three lots of ten each, and given the following treatment:

Lot 1, grown outdoors in normal day length.

Lot 2, grown outdoors for 9 hours each day and the remainder of the time in the dark room.

Lot 3, grown outdoors for 7 hours each day and the remainder of the time in the dark room.

Table I shows the effect of length of day on the type of roots produced, time of flowering, and the supply of nitrates present. Lots 2 and 3 were very much alike throughout, varying only in size. The normal day plants produced many more leaves and became larger in general than short day plants. Microchemical tests showed a very high supply of nitrates in the leaves of short day plants, while very little appeared in the normal day plants. The hydrogen-ion concentration of expressed sap from stems or leaves deviated only slightly from pH 5.4 in plants of all lots. The normal light plants developed mostly fibrous roots with only an indication of storage, while both

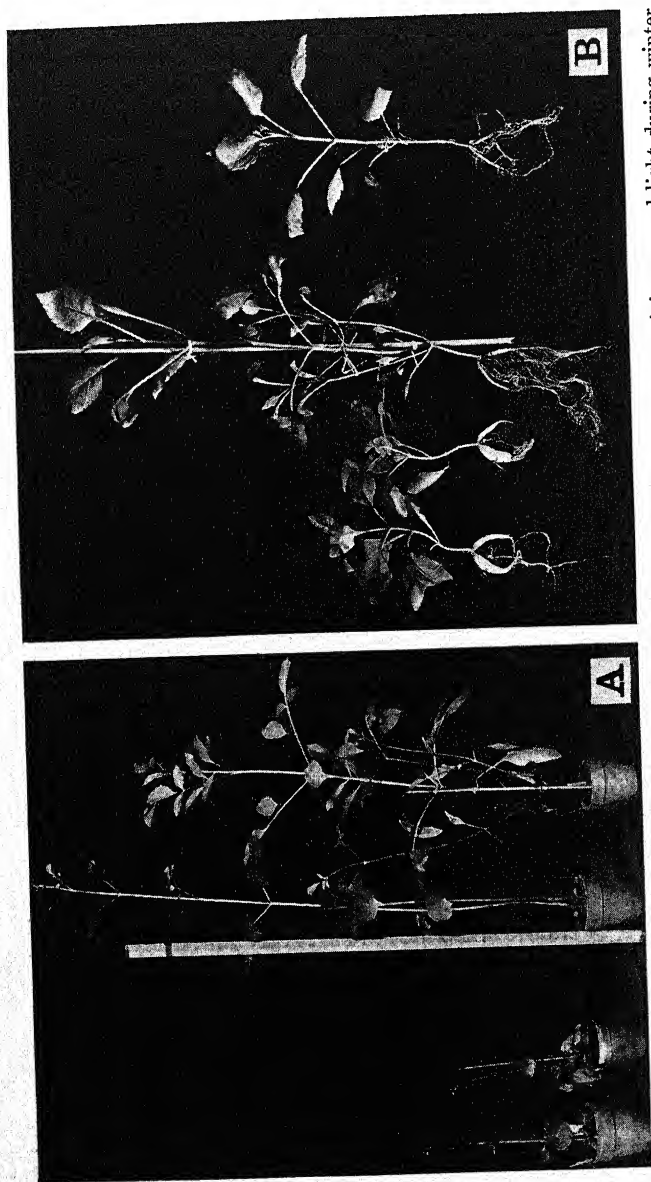


FIG. 3.—Seedling dahlias: *A*, grown from November 3 to February 7; left, plants receiving normal light during winter months flowered when only a few inches high; right, same as those on left in the beginning, but given extra illumination from sundown to midnight; *B*, grown from November 3 to January 4; two large plants with fibrous roots given extra light from sundown to midnight; small dahlias with storage roots received usual light of November and December: note that extra light almost completely eliminated formation of storage roots, while short day plants, although only a few inches high, produced unusually large storage roots.



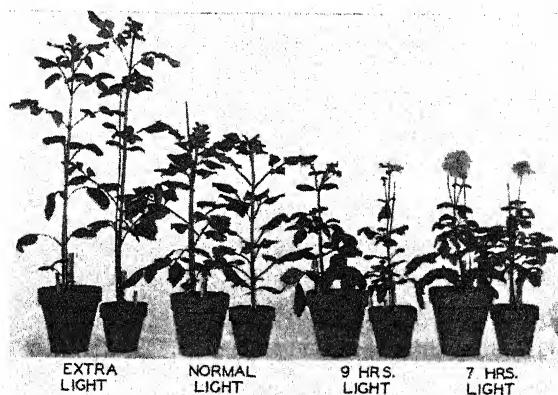


FIG. 4.—Top, *Dahlia variabilis* (Jersey's Beauty), June 8 to July 17, showing effect of length of day on top growth and flowering; plants in large pots had old storage root attached; plants in small pots were grown from cuttings; first flower buds occurred in short day plants on June 26, and on long day plants August 6; all short day plants had flowered by July 17; normal light plants started to flower August 20. Middle, root systems of plants from lots photographed in top row; extra light plants were omitted, otherwise the order is as appears in top row; photographed August 6. Note heavy root storage in short day plants; normal light plants developed fibrous roots with only a slight tendency to produce storage roots; when storage roots started, they were elongated and unlike the storage roots of short day plants. Below, Mrs. I. de ver Warner dahlias showing effect of length of day on roots and tops; labeled as in top row, started June 8 and photographed August 6; this variety responds almost exactly like Jersey's Beauty.



the 7-hour and 9-hour plants had large storage roots. Where storage roots had started on normal day plants they were slender, with long necks, whereas those on short day plants were short and rounded like potato tubers (fig. 4).

In order to find whether the presence of stored food material influenced the type of root system or the time of flowering, five plants in each lot were grown with old "tubers" attached. The response was essentially like that of cuttings (fig. 5).

TABLE I  
EFFECT OF LENGTH OF DAY ON TYPE OF ROOTS AND TIME OF FLOWERING OF JERSEY'S  
BEAUTY DAHLIA; DURATION OF EXPERIMENT JUNE 8 TO AUGUST 6, 1928

LIGHT TREATMENT	No. OF PLANTS USED		DATE OF FIRST VISIBLE FLOWER BUDS	No. OF PLANTS FLOW- ERING ON JULY 17	AVERAGE HEIGHT		TYPE OF ROOTS FORMED	NITRATES (MICRO- CHEMICAL TESTS)
	Cut- tings	With old "tu- bers"			Cut- tings	With old "tu- bers"		
Lot 1, normal day length.	5	5	August 6	0*	2'6"	3'	Fibrous roots with slight tendency to form storage roots	Nitrates low in leaves and stems
Lot 2, 9 hours of daylight.	5	5	June 28	10	1'6"	1'8"	Storage roots with com- paratively few fibrous roots	Nitrates abundant in leaves and stems
Lot 3, 7 hours of daylight.	5	5	June 26	10	1'4"	1'6"	Storage roots with com- paratively few fibrous roots	Nitrates abundant in leaves and stems

\* A few plants remaining after the experiment was discontinued showed flower buds on August 31.

In addition to Jersey's Beauty, six other named varieties have been used in experiments. Of these, four varieties (Mrs. I. de ver Warner, Trentonian, F.T.D., and Insulinda) formed storage roots and flowered only when given short day treatment. Table I, showing data for Jersey's Beauty, could almost exactly be duplicated for Mrs. I. de ver Warner. The main difference was a slightly earlier bud development in Warner plants. Arthur (pompon), Esther R. Holmes, Maid of Watts, and Summer Red were indifferent to length of day in respect to flowering, but storage roots were favored by short days (fig. 6).

There are many varieties of dahlia that flower throughout the long days of summer. The pompon types, particularly, are known

for this characteristic. Fig. 6 shows two pompons (Arthur) which flowered in both long and short days. They show, however, a great difference in root storage. The photograph showing roots was taken on August 8, after the days were beginning to shorten. Note that the long day plant has many fibrous roots, but carbohydrate storage had started. The short day plant, however, has much heavier storage. In this case it is clear that carbohydrate accumulation is independent of flowering, but is controlled by the length of day. Also flowering is independent of storage root formation, for in the long day plant, flowering had been accomplished before storage had started in the roots. In types like Jersey's Beauty, flowering and the development of storage roots took place concurrently in the short days. Long day plants could form neither storage organs nor flowers (fig. 5). It so happens that the same factor, the length of day, controls both responses at the same time in this type of dahlia, although root storage and flowering are probably not correlated in a causal way. This supposition is strengthened by the fact that plants grown with old storage roots attached responded to day length almost exactly as did cuttings (fig. 6). "Tubers" attached to the pompon dahlias did not change their flowering response, as these plants flowered under all conditions.

Temperature was not controlled in any of the experiments with dahlias. Possibly both light and temperature could be determining factors. In the case of potatoes, GARNER and ALLARD (5) showed that tuberization could be completely eliminated by extra illumination. BUSHNELL (3), SMITH (7), FITCH (4), and others completely eliminated tuberization of potatoes by keeping the temperature up to 29° C., while plants at 20° C. tuberized heavily. This suggests that work ought to be done in which both light and temperature are taken into consideration.

The effect of light on cuttings while they are in the rooting medium has long been a subject under discussion. The point of interest has usually been the effect of light intensity. In case of dahlia both light intensity and duration are important. Vegetative growth is so limited by short light duration that few or no fibrous roots can be produced. Such roots as can grow are storage in nature. Many variations from normal storage roots have been noted. Old stems



FIG. 5.—*Dahlia variabilis* (Jersey's Beauty) plants with old storage root attached, June 8 to August 6; left to right, normal light, 9 hours' light, and 7 hours' light: note that short day plants flower and produce storage roots the same as do plants grown from cuttings; there is no noticeable influence from the old storage root (indicated by black cross).



FIG. 6.—*Dahlia variabilis* [Arthur (pompon)]: A, plants from cuttings, June 8 to July 16; plant on left received normal light; plant on right received 9 hours of light; this variety flowers on both long and short day illumination; B, plants same as those photographed in A, but grown from June 8 to August 6: note that root types are same as in other varieties, although flowering is irrespective of light.

become modified at points where carbohydrates accumulate, and the lowest buds develop into tuber-like organs (fig. 2). Leaves placed in the rooting medium often formed roots, but whether they rooted or not, the basal end of the petiole became enlarged and took the place of storage tissue. Cuttings grown earlier in the season, when the days were longer, had no such tendency toward developing storage organs. Normal fibrous roots were the rule. There is doubtless a certain day length where both vegetative growth and storage can proceed at an even rate. On either side of this particular duration one would have an advantage over the other.

It is of interest to note that nitrates accumulate in the short day plants (table I). NIGHTINGALE (6) found that *Salvia*, buckwheat, and soy beans illuminated for only 7 hours each day had a higher percentage of nitrates and carbohydrates than the long day plants. He states that carbohydrates accumulate in the short day plants, presumably because there is relatively little utilization of them in the synthesis of nitrates to other forms of nitrogen. Even though growth is checked, if the plant remains in good condition, carbohydrates will continue to be manufactured and nitrates to be absorbed. It seems reasonable to suppose that when any growth process is interfered with, the utilization of the two main building materials, carbohydrates and nitrates, will correspondingly decrease, thus permitting their accumulation. In the case of the dahlia, a 7- or 9-hour day quickly brings vegetative growth to an end and flowering is accomplished. The plant remains in good condition thereafter for a considerable period of time, continuing to manufacture carbohydrates and absorb nitrates. As these two substances accumulate, the plants take on the appearance of approaching dormancy. They do pass, in time, into complete dormancy and lie for two or more months before they are able to grow. If they are changed early enough from short to long day, dormancy is prevented and vegetative growth starts.

### Summary

1. Length of day determines the type of root system formed by cuttings for 6 varieties of dahlia, heavy root storage being correlated with a short day, and a fibrous root system being correlated with a long day.

2. Flowering was found to be independent of storage root formation, although certain varieties, such as Jersey's Beauty and Mrs. I. de ver Warner, flowered and formed storage roots concurrently on a short day.

3. Certain varieties, such as Arthur and Summer Red, flowered independently of day length; while others, such as Jersey's Beauty and Warner, flowered only on a short day.

4. Cuttings taken during late September and October developed various types of storage organs along the stem without forming either typical storage roots or fibrous roots.

5. Nitrates accumulated in the leaves and stems of short day plants, but were absent or present in only small amounts in long day plants.

6. The hydrogen-ion concentration of stems or leaves of long and short day plants varied only slightly from pH 5.4.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.  
YONKERS, N.Y.

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## MOSAIC DISEASE OF TOBACCO. I. PROGRESS IN FREE- ING THE VIRUS OF ACCOMPANYING SOLIDS<sup>1</sup>

C. G. VINSON AND A. W. PETRE

### Introduction

BEIJERINCK (3) reported working with an infectious juice expressed from plants with mosaic disease. He obtained a precipitate with alcohol from the juice. This precipitate, after drying at 40° C., retained its infectivity. HEINTZEL (8) obtained the same results as BEIJERINCK. ALLARD (1), VINSON (16), and SMITH (15) have also reported the use of alcohol in precipitating the virus from juice of diseased plants. ALLARD (1) has shown that an active fraction can be adsorbed from juice of diseased plants by talc and aluminium hydroxide. He also obtained a glycerin extract from dried, ground leaf material which, after filtering, appeared to contain more of the original activity than the residue. VINSON (16), in addition to corroborating the observations on precipitation by alcohol, reported analogous behavior with acetone, ammonium sulphate, and safranin. BREWER, KRAYBILL, and GARDNER (4) reported activity in a colorless solution obtained by adsorbing the virus on charcoal, washing out the coloring matter, and then freeing the virus.

The biological reaction is the only test available, so far as known, for determining the presence of the virus. No distinction can be made as to the relative virus concentrations when the methods of inoculation in general use are employed. MCKINNEY (12) has called attention to the desirability of a method for determining virus concentrations, and has devised a method which detects considerable differences in virus concentration. The method of HOLMES (9), based on the introduction of a small dose, detects differences in virus concentration within more restricted limits.

This paper gives in detail the results obtained in removing the virus from infectious juice by methods previously described (16), and also reports further progress in freeing the virus of accompany-

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ing solids. We submit evidence, based on measurements obtained by an adaptation of HOLMES' technique, that the virus of mosaic disease of tobacco can be freed of 90 per cent of the non-active solids of the juice with no apparent loss in infective power.

### Preliminary work

Only diseased tomato plants were at first available. Leaves and tips of these plants were collected from the field. Part of this material was treated with ether (5) in the fresh condition, and the cell sap expressed at once with a hydraulic press. The other part of the plant material collected was placed in a cold room and allowed to freeze. It was then thawed and the cell sap expressed. Both lots of juice proved to be infectious.

On concentration in vacuo the juice frothed badly, especially that from the etherized plants. Caprylic alcohol and diphenyl ether were successfully used as foam breakers. A few drops of either of these substances reduced the tendency to froth considerably, but after their use the juice became inactive, even though the temperature of the water bath was never allowed to rise above 50° C. It was found, however, that juice from frozen plants could be concentrated in vacuo without troublesome frothing, the flask requiring close attention only when boiling began.

An attempt was made to remove some of the solutes present in the concentrated juice by electrodialysis. The first solution electro-dialyzed was one prepared by concentrating 2300 cc. of juice from frozen tomato plants to 150 cc. To the 150 cc. of concentrate 20 per cent, by volume, of ethyl alcohol was added as a preservative. This solution was infectious. Its pH was 4.76. In the electrodialysis work a Y tube was used, and the ends of the two arms covered with linen cloth impregnated with collodion. The arms on the Y were inserted into two separate small beakers of distilled water containing a trace of magnesium sulphate as electrolyte. Platinum electrodes were inserted in the beakers, consequently the electrodes did not come in contact with the concentrated juice. The Y tube was then filled up to the stem with the concentrated juice. The dialysis was allowed to run for about 18 hours, the current being about 10 milliamperes at 20 volts. When the experiment was terminated, samples were



taken from both of the beakers and both arms of the Y tube. That from the arm of the tube projecting into the beaker which contained the negative electrode was infectious. The other samples were not infectious. The experiment was repeated on a second portion of the same sample, and the same results were obtained. This indicated that the active principle, under certain conditions, carried an electric charge.

### Experimental work

#### PREPARATION OF MATERIAL

Tobacco plants were used in the following experiments. The original stock of virus was obtained from Professor JAMES JOHNSON,

TABLE I  
DATA SHOWING MOST OF VIRUS IS OBTAINED IN JUICE EXPRESSED FROM  
TISSUES FIRST FROZEN, THEN THAWED

DILUTION	EXPRESSED JUICE 700 CC.		FIRST AQUEOUS EXTRACT OF PRESS CAKE, 600 CC.		SECOND AQUEOUS EXTRACT OF PRESS CAKE, 604 CC.		THIRD AQUEOUS EXTRACT OF PRESS CAKE, 523 CC.	
	No. of plants inocu- lated	No. diseased	No. of plants inocu- lated	No. diseased	No. of plants inocu- lated	No. diseased	No. of plants inocu- lated	No. diseased
Undiluted.....	10	10	10	8	10	7	10	6
1/250.....	10	6	.....	.....	.....	.....	10	0
1/500.....	10	6	10	1	10	1	.....	.....
1/1000.....	10	6	10	2	.....	.....	.....	.....
1/5000.....	10	4	.....	.....	.....	.....	.....	.....
1/10000.....	10	2	.....	.....	.....	.....	.....	.....

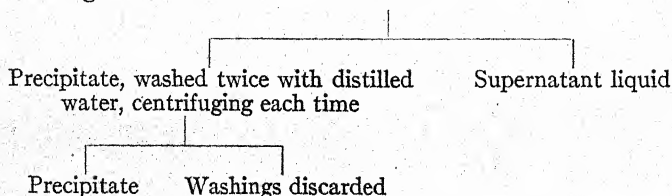
Department of Plant Pathology, University of Wisconsin. This virus gave the "marked mottling, malformation and stunting" characteristic of the mosaic disease of tobacco as found in the field. The juice samples on which our observations have been made were obtained from frozen diseased leaves, or from leaves and stalks after thawing at room temperature. The juice was pressed out of the thawed material (without grinding) at 500 pounds per square inch, then centrifuged for 10 minutes at 1500 r.p.m. This gives, in the centrifuge used, a centrifugal force about 600 times that of gravity. The juice obtained is brown, and free from large suspended particles. That obtained only from leaves contains about 22 per cent of the leaf solids and 12.5 per cent of the leaf nitrogen. That the juice obtained

by this method from diseased plants contains the greater portion of the virus is shown by the infectivity of the expressed juice, as compared with the aqueous extracts of the ground press cake. Results of tests of juice and extracts are given in table I.

#### PRECIPITATION OF VIRUS WITH AN AQUEOUS SOLUTION OF SAFRANIN

The results in electrodialysis, and the fact that ALLARD (1) was able to remove the virus from solution with talc, gave evidence that the virus possessed some properties characteristic of colloids. It was natural, therefore, to expect that certain substances which could neutralize the charge or affect the water relationship of the active principle might throw it out of solution. Solutions of certain dyes were therefore added to samples of juice from diseased tobacco plants. Salts of acid dyes such as crystal ponceau and rose bengal gave a precipitate when added to juice from diseased plants. That produced by rose bengal settled rapidly, but when the supernatant liquid was tested it was found to be active. Solutions of basic dyes such as Bismarck brown and safranin were tried. Bismarck brown gave a precipitate which settled rapidly, but the supernatant liquid retained activity. Ten cc. of a 1 per cent safranin solution when added to 50 cc. of juice from diseased plants gave a precipitate which flocculated and settled very slowly. The supernatant liquid was, according to our determinations, almost free from virus. Safranin, therefore, was the only one of these dyes found capable of precipitating the virus almost completely. It is to be expected, however, that other azine dyes will also precipitate the virus.

The safranin precipitate was obtained according to the following scheme. Juice from diseased plants was treated with a 1 per cent aqueous solution of safranin in the proportion 10 to 50 cc. of the juice, then placed in the ice box for several hours, usually overnight, and centrifuged:



## FREEING VIRUS FROM SAFRANIN PRECIPITATE

Safranin being a weak base, it was thought that by adding a small amount of acid or a small amount of stronger base to a suspension of the safranin precipitate, the virus might be set free. Table II gives the results obtained in preliminary attempts to free the virus from the precipitate by acid and alkali treatments. As indi-

TABLE II  
ATTEMPTS TO FREE VIRUS FROM SAFRANIN PRECIPITATE BY ACID AND  
ALKALI TREATMENT

EX- PERI- MENT NO.*	PREPARATION	TREATMENT	NO. OF PLANTS INOCU- LATED	NO. DISEASED
1..	(a) Precipitate suspended in 0.5 per cent agar solution	None	10	1
	(b) 15 cc. of suspended precipitate	+3 cc. N/14 HCl	5	1
	(c) 15 cc. of suspended precipitate	+3 cc. N/14 NaOH	5	0
	(d) 30 cc. of supernatant liquid	+3 cc. N/14 HCl	5	0
	(e) 30 cc. of supernatant liquid	+3 cc. N/14 NaOH	5	0
2..	(a) Precipitate suspended in 0.5 per cent agar solution	None	10	1
	(b) 25 cc. of suspended precipitate	+7 cc. N/10 acetic acid	10	0
	(c) 25 cc. of suspended precipitate	+5 cc. N/10 Na <sub>2</sub> CO <sub>3</sub> solution	10	0
	(d) Supernatant liquid	None	10	0
	(e) Supernatant liquid	Diluted 1 to 10	10	0
3..	(a) Precipitate suspended in 0.5 per cent agar solution	None	10	1
	(b) 25 cc. of suspended precipitate	+6 cc. N/10 acetic acid in cold	10	2
	(c) 25 cc. of suspended precipitate	+5 cc. N/10 Na <sub>2</sub> CO <sub>3</sub> in cold	10	4
	(d) Supernatant liquid	None	10	0
	(e) Supernatant liquid	Diluted 1 to 10	10	0
4..	(a) Precipitate suspended in dilute agar	None	10	1
	(b) 25 cc. of suspended precipitate	+5 cc. N/10 acetic in cold, pH 4.8	10	2
	(c) 25 cc. of suspended precipitate	+5 cc. N/10 Na <sub>2</sub> CO <sub>3</sub> in cold, pH 7.2	10	2
	(d) Supernatant liquid	None	10	0
	(e) Supernatant liquid	Diluted 1 to 10	10	1
5..	(a) Precipitate suspended in aqueous trypsin extract and let stand over-night in ice box	None	10	5
	(b) Precipitate suspended in aqueous pepsin solution 3 hours	None	10	5

\* The blanks used in these experiments were all healthy at the end of the respective tests.

cated in the table, these attempts were not successful. The dilutions of the supernatant liquid, 1 to 10, were made in order to reduce the concentration of the safranin to the point where it was known that it would not inhibit the activity of the virus. It was found by trial that juice from diseased plants treated at the rate of 1 cc. of a 1 per cent aqueous solution of safranin to 50 cc. of juice remains infectious. It is necessary to add an excess of the safranin solution to complete the precipitation.

ROBERTSON (14), HOLZBERG (10), and MARSTON (11) have shown that safranin forms a rather insoluble precipitate with proteoclastic enzymes. The safranin precipitate of the virus was treated, therefore, with a trypsin and also with a pepsin solution. By means of these treatments the virus was apparently displaced from its union with the safranin (see experiment 5, table II).

A means of removing the safranin from the safranin-virus precipitate, without inactivating the virus as it was set free, was then sought. After some preliminary tests it was found that picric acid forms a very insoluble precipitate with safranin. When 2 cc. of a saturated aqueous solution of picric acid is added to 25 cc. of a 0.16 per cent aqueous safranin solution, the precipitation is practically complete.

In attempts to free the virus from the safranin precipitate with picric acid, the following procedure was followed:

To 50 cc. of juice from diseased plants was added 15 cc. of a 1 per cent aqueous safranin solution; solution then placed in ice box several hours, preferably overnight, and centrifuged

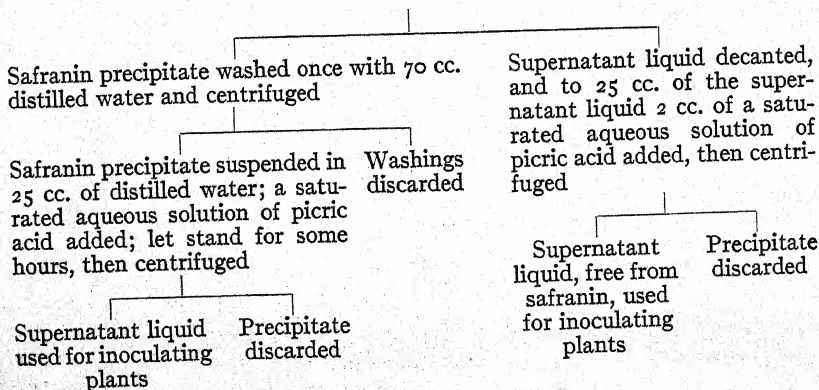


Table III gives the results obtained in freeing the safranin-virus precipitate from safranin. Experiment 5 indicates that the picric

TABLE III  
LIBERATION OF VIRUS FROM SAFRANIN PRECIPITATE BY MEANS OF PICRIC ACID

EX- PERI- MENT NO.	PREPARATION	PH OF SOLUTION	NO. OF PLANTS INOCU- LATED	NO. DISEASED
1..	(a) Safranin precipitate suspended in 25 cc. distilled water and 2 cc. saturated aqueous solution of picric acid added; after standing, solution centrifuged and supernatant liquid used for inoculating plants.....	3.9	10	4
	(b) 25 cc. of supernatant liquid from virus-safranin precipitate treated with 2 cc. of saturated aqueous solution of picric acid, centrifuged, and supernatant liquid, free of safranin, used for inoculating plants.....	5.5	10	0
2..	(a) Safranin-virus precipitate suspended in 25 cc. distilled water and 2 cc. saturated aqueous solution of picric acid added; after standing, solution centrifuged and supernatant liquid used for inoculating plants.....	4.3	10	3
	(b) 25 cc. of supernatant liquid from safranin-virus precipitate treated as in experiment 1..	5.3	10	0
3..	(a) Safranin-virus precipitate suspended in 25 cc. distilled water and 4 cc. saturated aqueous solution of picric acid and 1.2 cc. N/10 $\text{Na}_2\text{CO}_3$ solution added; after standing, solution centrifuged and supernatant liquid used for inoculating plants.....	3.7	10	3
	(b) 25 cc. of supernatant liquid from safranin-virus precipitate treated as in experiment 1..	5.2	10	0
4..	(a) Safranin-virus precipitate suspended in 25 cc. distilled water and 4 cc. picric acid and 2 cc. N/10 $\text{Na}_2\text{CO}_3$ solution added; after standing, centrifuged, and supernatant liquid used for inoculating plants.....	6.6	10	9
	(b) 25 cc. of supernatant liquid from safranin-virus precipitate treated as in experiment 1	5.3	10	2
5..	(a) To 25 cc. of diseased juice was added 2 cc. of saturated aqueous solution of picric acid, and solution used for inoculating plants.....	5.3	10	10

acid concentration used to remove safranin from the supernatant liquid of the virus-safranin precipitate does not affect the activity of the virus appreciably.

REMOVAL OF SAFRANIN FROM SAFRANIN-VIRUS  
PRECIPITATE BY MEANS OF AMYL ALCOHOL

On removal of the dye with normal amyl alcohol from the suspension of the safranin-virus precipitate, following dispersion in acid solution, activity is recovered in the aqueous layer. Hydrochloric, nitric, acetic, and oxalic acids have been used successfully to effect the dispersion of the precipitate and release the virus. When the volume of

TABLE IV  
LIBERATION OF VIRUS FROM SAFRANIN PRECIPITATE BY EXTRACTING  
SAFRANIN WITH AMYL ALCOHOL

EX- PERI- MENT NO.	PREPARATION	NO. OF PLANTS INOCU- LATED	NO. DISEASED
1..	(a) Aqueous layer, 50 cc. in volume, pH 5.8-6.0, after removing safranin from aqueous suspension of precipitate with amyl alcohol.....	10	10
	(b) Suspension of interfacial precipitate formed on removal of safranin with amyl alcohol.....	10	7
2..	(a) Safranin-virus precipitate suspended in 50 cc. H <sub>2</sub> O and 1 cc. N. HCl added; pH of resulting solution 2.9; safranin removed with amyl alcohol; aqueous layer, safranin free, but not neutralized.....	10	9
	(b) Same as 2(a), but neutralized.....	10	10
	(c) Same as in experiment 1(b).....	10	10
3..	(a) Safranin-virus precipitate suspended in 50 cc. H <sub>2</sub> O and 0.35 cc. N/1 HCl added; pH of resulting solution 3.1-3.2; aqueous layer, safranin free, but not neutralized..	10	10
	(b) Same as experiment 3(a), but neutralized.....	10	10
	(c) Same as in experiment 1(b).....	10	10
	(d) Supernatant liquid from safranin precipitate after removing safranin with amyl alcohol.....	10	3

water used to disperse the precipitate is twice the volume of the juice from which the precipitate is obtained, the addition of acid produces a clear transparent solution. Under these conditions the liberation of the virus from the dye appears to be optimal. The aqueous solution obtained following amyl alcohol washing is brown in color, resembling in appearance the original juice from diseased plants. A precipitate separates at the water-amyl alcohol interface; this precipitate is also active. The safranin-virus precipitate was obtained by adding 12 cc. of a 1 per cent aqueous safranin solution to 50 cc. of juice from diseased plants. Table IV gives the results obtained.

To discover whether the conditions for safranin precipitation would be more favorable in an alkaline medium, samples of juice from diseased plants were brought to a hydrogen-ion concentration of pH 7.2 by the addition of saturated calcium hydroxide solution. Twelve cc. of a 1 per cent safranin solution was then added to 50 cc. of the treated juice. The precipitate was removed by centrifuging,

TABLE V  
PRECIPITATING THE VIRUS WITH SAFRANIN FROM JUICE AT ALKALINE  
REACTION, THEN REMOVING THE DYE FROM PRECIPITATE AND  
SUPERNATANT LIQUID WITH AMYL ALCOHOL

EX- PERI- MENT NO.	PREPARATION	NO. OF PLANTS INOCU- LATED	NO. DISEASED
1..	(a) Safranin-virus precipitate suspended in 65 cc. H <sub>2</sub> O; pH of resulting suspension 7.7; aqueous layer, following removal of safranin, with amyl alcohol.....	10	10
	(b) Suspension of interfacial precipitate after washing twice with water.....	10	9
2..	(a) Virus-safranin precipitate suspended in 50 cc. water, 1 cc. N/1 HCl added; pH of resulting suspension 3.0; safranin free aqueous layer, not neutralized.....	10	10
	(b) Same as 2(a), but neutralized.....	10	10
	(c) Same as in experiment 1(b).....	10	10
3..	(a) Safranin-virus precipitate suspended in 50 cc. water and brought to pH 3.6 by adding N/1 HCl; aqueous layer, following removal of safranin with amyl alcohol, not neutralized.....	10	10
	(b) Same as in experiment 3(a), but neutralized.....	10	10
	(c) Same as in experiment 1(b).....	10	10
	(d) Supernatant liquid from safranin-virus precipitate after removal of safranin with amyl alcohol.....	10	2
	(e) Untreated diseased juice.....	10	9
	(f) Diseased juice diluted 1 to 1000.....	10	1

then washed with 50 cc. of distilled water. Table V gives the results obtained on removing the dye from the precipitate and also the supernatant liquid with amyl alcohol.

#### SALTING OUT EXPERIMENTS ON VIRUS OF TOBACCO MOSAIC

Salting out experiments were tried after it was found that an aqueous solution of safranin precipitated the virus, and also that under certain conditions the virus apparently will migrate in an electric field.



TABLE VI  
SALTING OUT THE VIRUS FROM JUICE OF DISEASED PLANTS

EX- PERI- MENT NO.	PREPARATION	NO. OF PLANTS INOCU- LATED	No. DISEASED
1...	(a) 34 gm. $(\text{NH}_4)_2\text{SO}_4$ added to 50 cc. juice from diseased plants at room temperature, centrifuged; precipitate dissolved in 20 cc. distilled water, and solution used for inoculating plants.....	5	2
	(b) Supernatant liquid diluted with five volumes of distilled water.....	5	0
2...	(a) 17 gm. $(\text{NH}_4)_2\text{SO}_4$ added to 25 cc. juice from diseased plants at about $0^\circ\text{C}$ . in cold; after agitating, precipitate filtered off and dissolved in 15 cc. distilled water, and solution used for inoculating plants.....	5	4
	(b) 5 cc. filtrate made up to 50 cc. and solution used for inoculating plants.....	5	0
3...	(a) 16.5 gm. $(\text{NH}_4)_2\text{SO}_4$ added to 25 cc. juice from diseased plants at about $0^\circ\text{C}$ . in cold; after agitating, precipitate filtered off and dissolved in 20 cc. distilled water and solution used for inoculating plants.....	5	4
	(b) 5 cc. filtrate made up to 50 cc. with distilled water, and solution used for inoculating plants.....	5	0
4...	(a) 60 gm. $\text{MgSO}_4$ added to 100 cc. juice from diseased plants; after standing overnight at room temperature, precipitate filtered out, washed several times with saturated solution of magnesium sulphate; precipitate dispersed by pulping the filter paper in 50 cc. of water. On straining the suspension through cheesecloth a greenish brown solution was obtained which with washings measured 145 cc. This solution used for inoculating plants $2\frac{1}{2}$ days after the salt added.....	10	9
	(b) Aliquot of the 145 cc. obtained in experiment 4(a) diluted 1 to 100.....	10	9
	(c) Aliquot of the 145 cc. obtained in experiment 4(a) diluted 1 to 1000.....	10	8
	(d) Aliquot of the 145 cc. obtained in experiment 4(a) diluted 1 to 10,000.....	10	3
	(e) Original filtrate from precipitate obtained in experiment 4(a) used for inoculating plants.....	10	1
5...	(a) 13 gm. $\text{Na}_2\text{SO}_4$ (anhydrous) added to 100 cc. of diseased juice; after standing 18 hours at room temperature the precipitate centrifuged out and washed several times with small quantities of a 0.3 saturated solution of sodium sulphate; precipitate then dispersed in 75 cc. of water, and solution used for inoculating plants.....	10	5
	(b) Supernatant liquid obtained in experiment 5(a) diluted 1 to 100 and used for inoculating plants.....	10	9
6...	(a) Experiment 5(a) repeated, only variation being that after the $\text{Na}_2\text{SO}_4$ was added the solution was held at $32^\circ\text{--}33^\circ\text{C}$ . for 18 hours.....	10	3
	(b) Supernatant liquid obtained from precipitate in experiment 6(a) diluted 1 to 100 and used for inoculating plants.....	10	9

On attempting to salt out material from the diseased juice at about  $0^{\circ}\text{C}$ ., it was found that an immediate precipitate was thrown out only near the saturation point with ammonium sulphate. It was also found that about 34 gm. of ammonium sulphate to 50 cc. of the juice from diseased plants was necessary to produce this precipitate.

Table VI gives the results of some of the experiments on salting out the virus. The plants used to test these solutions were inoculated by scratching three leaves of each plant with a sterilized needle, dropping on the solution used for inoculating, and rubbing it in with a clean cork stopper.

Juice from diseased plants when 25 per cent saturated with ammonium sulphate remained infectious. In the experiments reported in table VI the original supernatant liquid and filtrates were diluted therefore, as indicated, in order to bring the ammonium sulphate concentration below 25 per cent saturation. High concentration of magnesium sulphate is apparently quite effective in salting out the virus. A precipitate forms when juice from diseased plants is 0.3 saturated with sodium sulphate and allowed to stand. As shown in table VI, however, it is doubtful whether this precipitate contains an appreciable proportion of the virus. Work on salting out the virus was soon discontinued, as the precipitates obtained not only contained the excess salt, but also protein and much pigment.

#### PRECIPITATION OF VIRUS OF TOBACCO MOSAIC WITH ACETONE OR ETHYL ALCOHOL

Since ALLARD (2) had shown that strong acetone or alcohol rapidly killed the virus, the precipitation by these reagents was first tried out in a cold room in order to reduce as much as possible the injury to the virus. Table VII gives the results obtained.

The preliminary experiments on precipitation of the virus by acetone, as shown in table VII, indicated that a solution of the precipitate, thrown out of juice from diseased plants by two volumes of acetone, is highly infectious. But in order to determine more accurately the proportion of the virus precipitated by adding acetone in the ratio of two volumes to one of the diseased juice, a greater number of plants were inoculated than were employed in the preliminary experiments. The method of HOLMES (9) was employed for

TABLE VII  
PRECIPITATING THE VIRUS WITH ACETONE

EX- PERI- MENT NO.	PREPARATION	NO. OF PLANTS INOCU- LATED	NO. DISEASED
1..	(a) To juice from diseased plants was added an equal volume of acetone in cold; supernatant liquid decanted and precipitate suspended in distilled water; suspension used for inoculating plants.....	5	5
	(b) Supernatant liquid from above precipitation used to inoculate plants.....	5	1
2..	(a) To juice from diseased plants were added two volumes of acetone in cold; supernatant liquid decanted and precipitate rinsed with acetone, then absolute ether; precipitate suspended in distilled water and used for inoculating plants.....	5	5
	(b) Supernatant liquid passed through a hardened paper filter, and filtrate diluted 1 to 3.....	5	0
3..	(a) Same as in experiment 2(a).....	5	5
	(b) Same as in experiment 2(b).....	5	0
4..	(a) Same as in experiment 2(a).....	5	5
	(b) Same as in experiment 2(b).....	5	0
5..	(a) To 25 cc. juice from diseased plants was added 50 cc. acetone in cold; precipitate decanted and precipitate rinsed with acetone, then ether; precipitate suspended in 13.33 per cent acetone and used for inoculating plants.....	5	5
	(b) Supernatant liquid from precipitate passed through gravity filter and filtrate diluted 1 to 5 (until acetone concentration was about 13.33 per cent).....	5	1
6..	(a) Same as in experiment 5(a).....	5	5
	(b) Same as in experiment 5(b).....	5	0
7..	(a) Same as in experiment 2(a).....	5	5
	(b) Same as in experiment 2(b).....	5	0
8..	(a) Same as in experiment 2(a).....	10	10
	(b) Same as in experiment 2(b).....	10	1*
9..	(a) Same as in experiment 2(a).....	10	10
	(b) Same as in experiment 2(b).....	10	1*
10..	(a) Same as in experiment 2(a).....	10	10
	(b) Same as in experiment 2(b).....	10	0
11..	(a) Same as in experiment 2(a).....	10	10
	(b) Same as in experiment 2(b).....	10	3†
12..	(a) Same as in experiment 2(a).....	10	10
	(b) Same as in experiment 2(b).....	10	2
13..	(a) To juice from diseased plants were added three volumes of acetone in cold; supernatant liquid decanted and precipitate rinsed with ether; precipitate then suspended in distilled water and used for inoculating plants.....	10	10
	(b) Supernatant liquid passed through hardened filter paper, and filtrate diluted 1 to 3, then used for inoculating plants.....	10	0
14..	(a) Same as in experiment 13(a).....	10	10
	(b) Same as in experiment 13(b).....	10	0
15..	(a) Same as in experiment 13(a).....	10	10
	(b) Same as in experiment 13(b).....	10	0

\* One of the blanks was also diseased.

† Three of the blanks were also diseased.

inoculation, whereby only a small amount of virus is introduced by means of fine pins. Table VIII gives the results of these experiments.

From experiments 2-15 of table VII and 3, 4, 7, and 8 of table VIII it is evident that, so far as our present methods indicate, precipitation (under certain favorable conditions) of the virus from juice of diseased plants is, for all practical purposes, complete when two volumes of acetone or absolute alcohol are added to one volume of the juice. The juice obtained from tobacco plants with mosaic disease varies not only in virus content, but also in the content of other solids. It has been observed that when apparently complete precipitation is obtained, the precipitate formed, on adding the acetone or alcohol in the cold, will settle out of solution very quickly and collect in a viscous mass in the bottom of the container. This permits decantation of the supernatant liquid without loss of the precipitate, giving a sharp separation. Under certain conditions, which are not yet entirely clear, a light flocculent precipitate may be obtained which settles slowly. This lengthens the time of contact with the reagent before the supernatant liquid can be decanted. It is also very difficult to decant the supernatant liquid completely from such a precipitate, even after it has settled, without losing some of the precipitate.

Experiments 4 and 7, table VIII, were designed to demonstrate whether loss of activity could be averted by more complete recovery of a precipitate which settled slowly as flocculent material. In this experiment the precipitate was thrown down in a compact mass by centrifuging. The mother liquor was decanted completely without loss of precipitate. Recovery was about complete, in contrast to experiment 2, table VIII, where a light flocculent precipitate was obtained and was not centrifuged. A greater concentration of acetone or alcohol will induce more prompt settling of the precipitate. We prefer, however, to work with the lowest concentration that will produce complete precipitation, as the rate of inactivation must be higher, the higher the acetone or alcohol content. We prefer acetone to alcohol in precipitating the virus, as the precipitate settles much better from acetone solution, and can be redispersed more readily in water.

TABLE VIII

COMPARISON OF AMOUNT OF VIRUS IN ACETONE OR ALCOHOL PRECIPITATE  
WITH THAT IN ORIGINAL JUICE SAMPLE

EX- PERI- MENT NO.	PREPARATION	NO. OF PLANTS INOCU- LATED	NO. DISEASED
1...	(a) 35 cc. juice from diseased plants made up to 100 cc. with C.P. acetone at room temperature, and allowed to stand a few minutes; precipitate obtained on centrifuging dispersed in water, made up to 100 cc., and solution used for inoculating plants. ....	150	54
	(b) 35 cc. juice from diseased plants used in 1(a) diluted to 100 cc. ....	150	74
2...	(a) 100 cc. juice from diseased plants, at about 0° C., added 200 cc. of C.P. acetone at -15° C.; precipitate did not settle, so about 50 cc. more acetone added, then supernatant liquid decanted; precipitate suspended in water and made up to 100 cc.; 50 cc. of this solution diluted to 100 cc. and used for inoculating plants. ....	150	31
	(b) 50 cc. of juice from diseased plants used in 2(a) diluted to 100 cc. ....	150	60
3...	(a) To 100 cc. of juice from diseased plants, at about 0° C., 200 cc. of C.P. acetone at about -15° C. was added; on mixing, precipitate settled at once, giving good separation; supernatant liquid decanted, precipitate suspended in water and made up to 100 cc.; 50 cc. of this solution diluted to 100 cc. and used for inoculating. ....	150	58
	(b) Diseased juice diluted with equal volume of water and used for inoculating. ....	150	51
4...	(a) Two volumes of C.P. acetone at about -15° C. added to juice from diseased plants at about 0° C. (last few cc. of mother liquor removed by centrifuging); precipitate taken up in water and diluted to a volume twice that of juice from which precipitate had been obtained. ....	150	60
	(b) Experiment 4(a) repeated on another sample of same juice. ....	150	64
	(c) Sample of same juice used in 4(a) and 4(b) diluted with an equal volume of water. ....	150	69
5...	(a) 30 cc. of juice from diseased plants made up to 100 cc. with 95 per cent alcohol; precipitate obtained after 30 minutes at room temperature dispersed in water and made up to 200 cc. ....	150	43
	(b) 30 cc. of the untreated juice made up to 200 cc. with distilled water. ....	150	58
6...	(a) 30 cc. of juice from diseased plants made up to 100 cc. with 95 per cent alcohol at room temperature; precipitate thrown down by centrifuging, then dispersed in about 30 cc. of 0.05 N. HCl (pH of dispersion 4.9). ....	150	45
	(b) Undiluted juice from diseased plants. ....	150	91
7...	(a) 33 cc. of juice from diseased plants at about 0° C. made up to 100 cc. with absolute alcohol at about -15° C.; precipitate thrown down by centrifuging a few minutes at room temperature; precipitate dispersed in 33 cc. of 0.025 N. HCl, then 33 cc. of 0.025 N. NaOH added (resulting pH 5.6r). ....	150	55

TABLE VIII—*Continued*

EX- PERI- MENT NO.	PREPARATION	NO. OF PLANTS INOCU- LATED	NO. DISEASED
	(b) Same preparation as 7(a) excepting that precipitate was dispersed in 66 cc. of 0.025 N. HCl (resulting pH 4.8)...	150	68
	(c) Same preparation as that of 7(a) excepting that precipitate was dispersed in 66 cc. of distilled water (resulting pH 6.2).....	150	52
	(d) Sample of same juice from diseased plants diluted with equal volume of distilled water (resulting pH 5.5).....	100	29
8...	(a) 33 cc. of juice from diseased plants made up to 100 cc. with absolute alcohol, then let stand in ice box 30 minutes; precipitate thrown down by centrifuging, washed with ether, then dispersed in 66 cc. of 0.025 N. HCl.....	150	55
	(b) Same preparation as that of 8(a) excepting that precipitate was dispersed in 66 cc. distilled water.....	150	76
	(c) Sample of untreated juice from diseased plants diluted with equal volume of distilled water.....	150	75

#### HEAT PRECIPITABLE FRACTIONS IN JUICE FROM DISEASED PLANTS

The work on salting out and precipitation with acetone and alcohol made it necessary to determine, if possible, whether simple proteins were connected in any way with the virus. It was soon found that juice from diseased plants contained a fraction that coagulated at about 85° C. When the temperature is raised at the rate of about 1° C. per minute in a water bath, the juice may become turbid near 70° C. The precipitate usually begins to flocculate at 85° C. or sometimes a little below, and the coagulum begins to settle before 90° C. is reached. This coagulum may readily be filtered off. It is very dark in color, and contains considerable nitrogen. The filtrate when heated to boiling gives another precipitate, but this contains only a small amount of nitrogen. Juice from healthy plants also contains these two heat precipitable fractions, but the first fraction may come down at a lower temperature than that from juice of diseased plants.

Table IX gives a comparison of the heat precipitable fractions from juice of healthy and diseased plants. Each precipitate was washed thoroughly by placing in a beaker and boiling with 100 cc. distilled water, filtering, and then repeating the operation once. As



shown from the table, there appears to be no marked difference in the nitrogen content of the two heat precipitable fractions from juice of diseased and healthy plants.

The juice remains infectious after the first fraction is removed by heating, although the virus strength is reduced. Boiling the juice renders it non-infectious.

#### PARTIAL REMOVAL OF PROTEIN, SULPHATE, AND PHOSPHATE

The acetone precipitate formed by adding two volumes of acetone to one volume of the juice from diseased plants contains from 20 to 25 per cent of the dry matter of the juice. This precipitate

TABLE IX  
HEAT PRECIPITABLE FRACTIONS IN JUICE FROM DISEASED AND HEALTHY  
TURKISH TOBACCO PLANTS

PREPARATION	PRECIPITATE OBTAINED BY HEATING TO 85° C.		PRECIPITATE OBTAINED BY BOILING FILTRATE, FROM FIRST PRECIPITATE, FOR 15 MINUTES	
	Weight (gm.)	Total nitrogen (gm.)	Weight (gm.)	Total nitrogen (gm.)
500 cc. juice from diseased tobacco plants.....	0.299	0.035	0.114	0.005
500 cc. juice from healthy tobacco plants.....	0.276	0.033	0.189	0.004

contains the protein fraction coagulating around 85° C., as well as much inorganic sulphate and phosphate. In beginning the work of reducing the dry matter content of the juice, and freeing the virus from more contaminating material, the first point of attack was the protein fraction just mentioned. Fractional precipitation with acetone was unsuccessful in separating the protein from the virus, as 25, 30, 35, 40, and 45 per cent acetone concentration failed to remove either all of the protein or a major part of the virus. It was finally found that most of the protein could be removed with basic lead acetate at a concentration which apparently left the virus unharmed. The solution employed was made by dissolving 200 gm. of Horne's basic lead acetate preparation in 1000 cc. distilled water. This solution is added at the rate of 19 cc. to 500 cc. of the juice from diseased plants. Extensive inoculation experiments have demonstrated that



this concentration of the lead acetate apparently does not reduce the virus concentration; but it does remove most of the protein, some phosphate, and, fortunately, much pigment. After treatment with lead acetate it was found that the acetone precipitate from the cleared juice contained considerable phosphate and sulphate. Barium acetate was then used to remove some sulphate and more phosphate. As much as 10 cc. of a saturated aqueous solution of barium hydroxide with 4 cc. of N. acetic acid have been added to 25 cc. of the juice without apparently destroying a great proportion of the virus. Following the clearing with lead, 20 cc. of a saturated aqueous solution of barium hydroxide with 8 cc. of N. acetic acid were added to 500 cc. of the original juice. Freshly expressed juice from diseased plants has a hydrogen-ion concentration of pH close to 7.0 following lead clearing; consequently, it is necessary to add acid with the barium hydroxide in order to keep the pH on the acid side. The use of 8 cc. of N. acetic acid with 20 cc. of the saturated barium hydroxide solution at 20° to 22° C. will bring the hydrogen-ion concentration of the juice to about pH 6.4 to 6.7.

It has been the practice to allow the juice to stand in a cold room overnight to freeze, following addition of the basic lead acetate solution, and also following the precipitation with an acid solution of barium acetate. In this way solutions showing only slight opalescence are obtained.

A number of preliminary experiments, using sets of ten plants each, and inoculating by the usual method of scratching the leaf surface and then rubbing in the liquid used for inoculating, gave just as many plants diseased from juice that had been treated with the lead and barium solutions as were obtained by inoculating with a sample of untreated juice. To estimate the amount of virus in this fraction the method of HOLMES (9) was employed, and table X gives the results obtained.

#### PRECIPITATION OF VIRUS FROM JUICE TREATED WITH LEAD ACETATE AND THEN BARIUM ACETATE

After precipitation with basic lead acetate and barium acetate, a flocculent precipitate forms when two volumes of acetone are added to one volume of such treated juice, at about 0° C. This precipitate

settles slowly and never permits complete decantation of the supernatant liquid without loss. After concentrating the treated juice (in vacuo with the bath at 50° C.) to 0.4 of the volume of the original juice taken, a precipitate is obtained when two volumes of acetone are added to one volume of the concentrated solution. This precipitate collects and settles at once, adhering to the bottom of the container. The supernatant liquid may then be decanted immediately

TABLE X

CLEARING JUICE FROM DISEASED TOBACCO PLANTS BY TREATMENT WITH  
LEAD ACETATE AND BARIUM ACETATE

EX- PERI- MENT NO.	PREPARATION	NO. OF PLANTS INOCU- LATED	NO. DISEASED
1..	(a) To 25 cc. juice from diseased plants was added 0.95 cc. of Horne's basic lead acetate solution* and centrifuged; to the supernatant liquid from basic lead acetate precipitation was added 1 cc. of saturated aqueous solution of barium hydroxide with 0.4 cc. N. acetic acid and centrifuged; aliquot from the supernatant liquid, following precipitation with Ba(OH) <sub>2</sub> , diluted with equal volume of distilled water and solution used for inoculating plants .....	150	80
	(b) Sample of untreated juice, same as used in preparing 1(a), diluted with equal volume of distilled water and this used for inoculating plants .....	150	60
2..	(a) Same as in experiment 1(a), except that another sample of juice from diseased plants was used .....	150	57
	(b) Same as in experiment 1(a), using aliquot of untreated juice from same sample as that used in preparing 2(a) ..	150	50

\* See text for description.

and drained off completely. More dry matter is eliminated in the latter than in the former case.

Each step in the procedure toward freeing the virus fraction from other solids has been checked numbers of times by the rough method of inoculation previously mentioned, using ten plants in each test. We have tested the activity of the acetone precipitate, from treated juice after concentration, only once by the method of HOLMES (9). Table XI gives the results of this experiment.

The experiment in table XI supports the results obtained with the rougher method. This experiment gives more evidence to the effect that there is little or no loss of virus accompanying precipita-

tion with basic lead acetate, followed by precipitation with barium acetate. For some time we were doubtful as to the effect on the virus

TABLE XI  
PRECIPITATING VIRUS WITH ACETONE FOLLOWING CLEARING OF JUICE WITH  
LEAD ACETATE AND BARIUM ACETATE

PREPARATION	NO. OF PLANTS INOCU- LATED	NO. DISEASED
19 cc. of basic lead acetate solution added to 500 cc. juice from diseased plants, mixed, placed in cold room overnight; next morning thawed, centrifuged		
Supernatant liquid, decanted, then added 8 cc. of N. acetic acid in 20 cc. saturated aqueous solution of barium hydroxide, mixed, then placed in cold room again overnight; next morning thawed, centrifuged	Precipitate discarded	
Supernatant liquid, decanted into distilling flask and concentrated to little below 200 cc.	Precipitate discarded	
Concentrated, decanted into beaker and made up to 200 cc.; placed in cold room and when freezing 400 cc. of C.P. acetone added (redistilled over soda lime) at about $-15^{\circ}$ C.; precipitate falls to bottom at once; supernatant liquid decanted completely	Distillate discarded	
Precipitate suspended in about 40 cc. of distilled water; centrifuged	Supernatant liquid discarded	
Supernatant liquid decanted, and made up to 50 cc.	Slight amount of sediment discarded	
1.25 cc. of this solution made up to 25 cc. with distilled water, and solution used for inoculating plants (this volume represents a dilution of original juice with equal volume of water).....	150	71
Aliquot from same original juice diluted with equal volume of water, and solution used for inoculating plants.....	150	68

of concentrating in vacuo below  $50^{\circ}$  C., but now it seems that the loss here also is small.

#### NITROGEN, DRY MATTER, AND ASH CONTENT OF VARIOUS FRACTIONS

To determine the effect of lead and barium treatment, and precipitation with acetone on the nitrogen, dry matter, and ash content of the fraction containing the virus, the determinations shown on pages 34 and 35 were made, and are representative of the results we have obtained.

The figures in the procedure outlined show that the acetone precipitate obtained, after lead and barium treatment, contains about 10 per cent of the dry matter of the original juice. Present experience indicates that the nitrogen content of juice from diseased plants exceeds that from healthy plants; while the dry matter of juice from healthy plants usually exceeds that from juice of diseased plants. In our system of fractionation, only samples of juice from diseased plants give the immediate precipitate with acetone, following concentration to 0.4 the volume of the original juice sample after lead and barium treatment. No sample of juice from healthy plants has been found to give the characteristic precipitate with acetone at this point in the procedure. A turbidity forms when the two volumes of acetone are added to the concentrated solution, following lead and barium treatment of healthy juice; but an appreciable precipitate settles to the bottom of the beaker only at the end of 10 or 15 minutes.

Preliminary experiments in inoculation, after treatment of the sample with hydrogen sulphide and following a second precipitation with acetone, indicate that the solutions obtained are quite active. We have not, however, made a quantitative study by the method of HOLMES at this point in the procedure.

Preliminary experiments indicate that a solution of the second acetone precipitate is inactivated when incubated with trypsin, or more especially by a combination of trypsin and pepsin; pepsin alone seems to have no effect on the virus.

#### Discussion

The present concepts of the nature of the virus of mosaic disease of tobacco are based, in part, on the following: the resistance to inactivation in 2 per cent phenol solution, 2 per cent creolin solution,

Juice from diseased plants, freshly expressed:

	gm.
Total nitrogen per 500 cc.....	0.252
Dry matter per 500 cc.....	12.172
Ash per 500 cc.....	5.650

500 cc. of this juice plus 19 cc. of Horne's basic lead acetate solution placed in cold room overnight, thawed, centrifuged

Supernatant liquid, decanted, and added 8 cc. of N. acetic acid in 20 cc. of saturated aqueous solution of barium hydroxide; placed in cold room overnight, thawed, centrifuged	Precipitate discarded
---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	-----------------------

Supernatant liquid, decanted into distilling flask and concentrated in vacuo to a little less than 500 cc.	Precipitate discarded
------------------------------------------------------------------------------------------------------------	-----------------------

Concentrate, decanted into 500 cc. volumetric flask and made up to the mark (excess lead not removed here)	Distillate discarded
------------------------------------------------------------------------------------------------------------	----------------------

420 cc. concentrated to little less than 140 cc. in vacuo	80 cc. used for analyses:	gm.
	total nitrogen in the 500 cc....	0.177
	dry matter in the 500 cc.....	10.365
	ash in the 500 cc.....	4.376

Concentrate, placed in cold and when freezing two volumes of C.P. acetone added at $-15^{\circ}\text{C}$ .	Distillate discarded
------------------------------------------------------------------------------------------------------------	----------------------

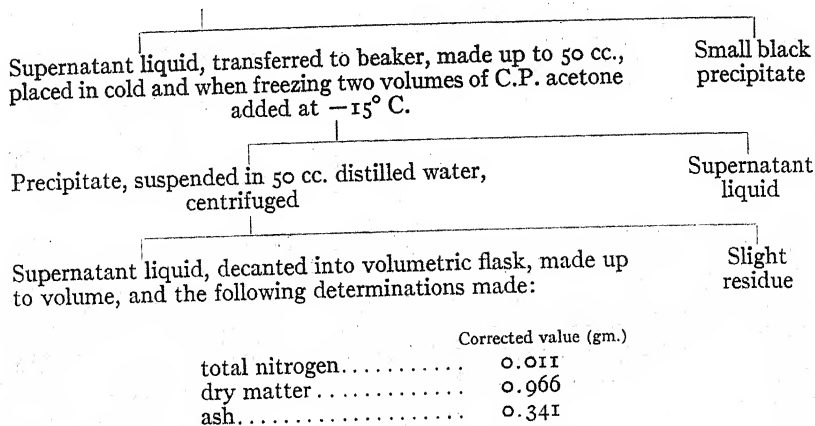
Precipitate suspended in 50 cc. distilled water, centrifuged	Supernatant liquid discarded
--------------------------------------------------------------	------------------------------

Clear supernatant liquid, transferred to 200 cc. volumetric flask and made up to volume	Slight residue
-----------------------------------------------------------------------------------------	----------------

120 cc. + $\text{H}_2\text{S}$ centrifuged	80 cc. used for analyses:	Corrected value (gm.)
	total nitrogen.....	0.018
	dry matter.....	1.343
	ash.....	.469

Supernatant liquid, concentrated in volume to less than 50 cc.	Small black precipitate
----------------------------------------------------------------	-------------------------

Concentrate, placed in cold overnight, then thawed, centrifuged	Distillate
-----------------------------------------------------------------	------------



and 1 per cent phenoco solution (2) is greater than that of vegetative forms of pathogens. The retention of activity in 50 per cent alcohol and 40 per cent acetone, accompanied by a complete loss of activity in higher concentrations (2), indicates less stability toward these reagents than cultures containing spores of *B. subtilis* (6), which after ten days in 99 per cent alcohol or 60 per cent acetone exhibit undiminished viability. Recently MULVANIA (13) has reported that the virus is not completely inactivated when exposed to ultra violet light 30 to 45 minutes; and a faint trace of activity remains after exposure to sunlight for 36 hours. This compares with a period of one to five minutes ultra-violet irradiation and one to two hours' exposure to sunlight required to kill bacteria either in vegetative or spore form. MULVANIA has summarized data to show that the virus of mosaic disease of tobacco is inactivated by a temperature intermediate between the thermal death point of vegetative and spore forms of pathogens.

WOODS (17) was the first to suggest that the virus of the mosaic disease of tobacco was enzymic in nature. FREIBERG (7), after reviewing the evidence available on its precipitation by alcohol, adsorption on talc and inactivation by high concentrations of alcohol, by low concentrations of formaldehyde and at high temperatures, also expressed the view that the active agent was an enzyme.

We have found that when precipitation of the virus is carried

out under favorable conditions, with the proper concentration of safranin, acetone, or ethyl alcohol, the precipitation is almost complete. In each case the precipitate contains practically all of the original activity of the juice, and the virus concentration in the supernatant liquid is no greater than that obtained by diluting a fresh juice sample one thousand-fold. This, together with the fact that the virus is apparently held in an inactive condition in the safranin precipitate and is released when the safranin is removed, makes it probable that the virus which we have investigated reacted as a chemical substance.

### Summary

1. An aqueous solution of safranin precipitates the virus of tobacco mosaic from juice of diseased plants. This precipitate brings down practically all of the virus. The virus is apparently held in an inactive condition in the precipitate, but is released when the safranin is removed by means of amyl alcohol.
2. Material, which gives an infectious solution when redissolved in water, has been salted out of infectious juice with ammonium sulphate and also with magnesium sulphate.
3. Two volumes of acetone or alcohol, when added to one volume of juice from diseased plants, at about  $0^{\circ}$  C., throws down a precipitate which contains practically all of the virus.
4. Juice from Turkish tobacco plants with mosaic disease contains two well defined heat precipitable fractions. One comes down around  $85^{\circ}$  C. and the other above  $90^{\circ}$  C. Only the first fraction contains an appreciable amount of nitrogen. The juice remains infectious after removal of the first fraction, although the virus concentration is greatly reduced.
5. Some of the phosphate, sulphate, and most of the protein and pigment may be removed, from juice of diseased plants, with low concentrations of lead acetate and barium acetate without apparently removing or injuring the virus.
6. Juice from diseased plants, after clearing with lead acetate and barium acetate, may be concentrated in vacuo, below  $50^{\circ}$  C., without apparent injury to the virus.
7. Cleared juice from diseased plants concentrated in vacuo to



0.4 the original volume and brought to about 0° C. gives a precipitate when two volumes of acetone at -15° C. are added. This precipitate contains only about 10 per cent of the solids of the original juice, but apparently contains all of the original virus.

8. The behavior of the virus is in many ways analogous to that of a chemical substance.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.  
YONKERS, N.Y.

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## LOCAL LESIONS IN TOBACCO MOSAIC<sup>1</sup>

FRANCIS O. HOLMES

(WITH ELEVEN FIGURES)

### Introduction

The literature concerned with the virus diseases of plants repeatedly emphasizes the systemic nature of these infections. MAYER (6), in his original description of tobacco mosaic in 1886, stated that symptoms do not develop on the leaf inoculated, but appear on all of the young developing leaves. Essentially the same account of the course of the disease was given by BEIJERINCK (2) in 1898, by IWANOWSKI (5) in 1903, by ALLARD (1) in 1914, and by others in more recent years.

No detailed descriptions of local lesions developing at the points where tobacco mosaic virus has been introduced have been published. This has probably been due in part to the fact that the local lesions are not conspicuous in commercial tobacco, *Nicotiana tabacum*, which has been used extensively. Another factor which has made the recognition of the local development of the disease more difficult has been the practice of inoculation by scratching and severe wounding. This tends to obscure the primary lesions by producing dead areas mechanically.

A few references in the literature indicate that local lesions have been observed, although their real nature has not been understood. ALLARD (1) referred to *N. langsdorffii* as follows:

Plants of this species when inoculated through the stalk and petioles seem particularly susceptible to a very destructive and progressive rot, which begins at the point of inoculation and finally kills the plant by slowly involving the surrounding tissues. *Nicotiana viscosum* (*N. glutinosa*) is sometimes killed in exactly the same manner.

FERNOW (3) said of *N. rustica*: "The leaves generally turn yellow and then brown near each point of inoculation." The briefness of

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

these references to the local lesions developing at the site of inoculation with tobacco mosaic virus indicates that these lesions were not recognized as symptoms of the disease.

There is in the literature one account of a plant virus which produces lesions at the point of inoculation. This is the virus of ring-spot of tobacco, which PRIODE (7) describes as forming typical rings of necrotic tissue around needle punctures used to introduce it into tobacco and petunia. These local lesions are exactly like the lesions later produced when the disease becomes systemic; but tobacco mosaic has never been shown to act in this way. Most writers have specifically stated or implied that the inoculated leaf if fully developed never shows symptoms of the infection.

In the present paper the local lesions caused by the introduction of tobacco mosaic virus into the tissues of a number of *Nicotiana* species will be described, and the usefulness of these lesions in measuring the concentration of mosaic virus samples will be shown.

In a former paper (4) the writer has described a method of inoculating plants by means of very small needle punctures. This method is well suited to the demonstration of the occurrence of local lesions. The absence of extensive dead tissue around such needle punctures when transmission does not take place makes the slightest deviation from the normal condition of the leaf tissues conspicuous. The present study was undertaken because evidence was obtained that changes take place at the site of inoculation of tobacco mosaic virus into *N. tabacum*. In this plant it was noticed that pale yellow areas sometimes develop around one or more of the five pin pricks made in each plant. Since in some cases a very dilute virus was being measured, not many plants were expected to take the disease. This fact made the pale yellow areas particularly noticeable, and led to a later examination of the plants which showed them. It was found that every plant which showed such local changes around one or more of the pin pricks developed mottling within a few days. Most of the plants which did not show pale yellow areas near the inoculation punctures remained healthy. This suggested that these yellow areas might represent localized symptoms of a primary infection, since they were consistently followed by the familiar systemic symptoms. Unfortunately the pale yellow areas were very incon-

spicuous and could not be detected in all cases in which systemic symptoms developed.

It seemed possible that some species of *Nicotiana* might show local symptoms more conspicuously and more consistently than *N. tabacum*. A survey of a number of species<sup>2</sup> was therefore made. These were *N. rustica*, *N. trigonophylla*, *N. plumbaginifolia*, *N. longiflora*, *N. tomentosa*, *N. suaveolens*, *N. quadrivalvis*, *N. paniculata*, *N. sylvestris*, *N. sanderae*, *N. glutinosa*, *N. nudicaulis*, *N. langsdorffii*, *N. clevelandii*, *N. acuminata*, *N. glauca*, and *N. multivalvis*. Of these species, five showed pronounced necrotic local lesions instead of pale yellow areas. These species were *N. rustica*, *N. langsdorffii*, *N. acuminata*, *N. sanderae*, and *N. glutinosa*. A description of the local lesions in each of these five species follows.

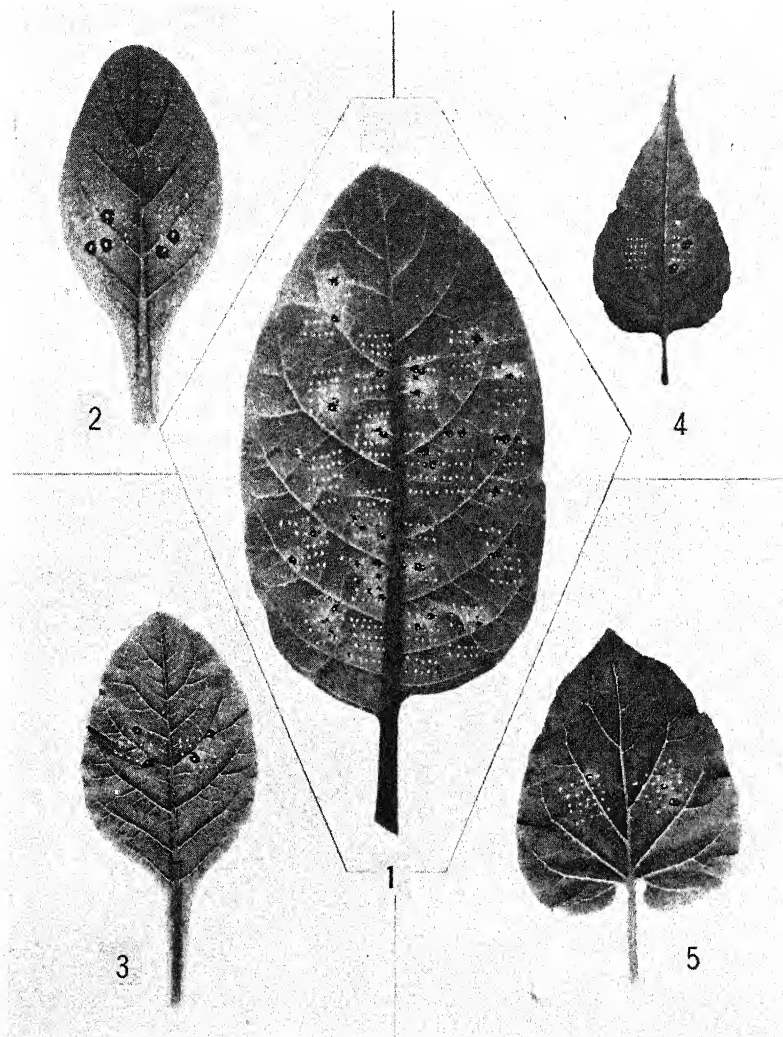
In *N. rustica*, as in all of the species studied, pin pricks which fail to transmit the virus heal perfectly with no macroscopic trace of necrosis; successful transfers, on the other hand, are marked by necrotic spots. These are easily counted eight to ten days after the time of inoculation, and may be distinguished even when many are on the same leaf (fig. 1). These necrotic spots on *N. rustica* are brown in color, circular in some cases, but frequently irregular in outline. They increase slowly in size during the first week after they appear. Later they spread rapidly, especially in young leaves. They may involve a considerable area of the leaf surface before the leaf concerned becomes old and drops off.

The lesions on *N. langsdorffii* (fig. 2) and *N. sanderae* (fig. 3) are more conspicuous than those on *N. rustica*, because they are blackish with concentric rings of dead tissue. They grow larger day by day after their first appearance, and may become very extensive. Sometimes veins and even the stem of the plant are involved in the slowly spreading necrosis of the tissues.

In *N. acuminata* (fig. 4) the lesions are irregular in outline and brown in color. They appear one or two weeks after the introduction of the virus, and increase in size rather more slowly than is the case in the other species.

<sup>2</sup> Thanks are due to Dr. S. A. WINGARD of the Virginia Agricultural Experiment Station, Dr. R. E. CLAUSEN of the University of California, and Dr. E. M. EAST of the Bussey Institution, Forest Hills, Massachusetts, for their kindness in supplying seeds of these species.

*N. glutinosa* exhibits a very different response (fig. 5). The lesions begin to be noticeable on the second or third day and are all completely formed by the fifth day, when in other species the



FIGS. 1-5.—Local necrotic lesions in: (1) *N. rustica*, (2) *N. langsdorffii*, (3) *N. sanderae*, (4) *N. acuminata*, (5) *N. glutinosa*. Lesions in *N. rustica* and *N. acuminata* are brown, in *N. langsdorffii* and *N. sanderae* almost black, and in *N. glutinosa* pale brown surrounded by rings of darker brown.

first indications of breakdown appear. These rapidly developing necrotic lesions appear first as tiny glistening dark spots. The centers soon dry down and develop a light brown color. Around them darker brown rings form concentrically. This gives the lesions an easily recognized appearance not to be confused with other dead spots in the leaves.

In order to discover whether these necrotic local lesions consistently appear whenever a successful transfer of the mosaic virus is accomplished, a test with small *N. rustica* plants was arranged. In a number of experiments each plant was inoculated with a single pin prick in a young leaf. The inoculation was carried out with a no. 00 black enamel insect pin previously wet with juice from a mosaic leaf of commercial tobacco. Altogether 582 plants were thus inoculated. Of these, 136 took the disease, as shown by the development of the severe systemic symptoms characteristic for this species. Every one of these plants showed a local necrotic lesion at the site of inoculation. These lesions were similar to those shown in fig. 1, except that there was but one on each plant. The fact that every plant which took the disease showed a local lesion is evidence that the local lesion is a definite symptom of the mosaic. Six plants in the series showed local lesions without a later development of the systemic disease. Apparently the virus was not able to spread in these individual cases. Their number is so small, however, that the diagnosis of transfer of the disease is not much changed if local lesions are taken as evidence of the transfer without waiting to examine the later systemic symptoms. Later experiments, in which more than one pin prick was used for each plant, have substantiated these conclusions.

Further evidence that the successful transfer of virus can be detected by examination of the local necrotic lesions is afforded by the fact that concentrated virus samples cause the development of large numbers of these local lesions when they are used as inoculum, whereas known dilutions of the virus samples in water cause the development of smaller numbers of the lesions. Graphs showing the accuracy with which the numbers of lesions are dependent upon the known concentrations of virus samples will be presented later in this paper, when the use of the local lesions in measuring virus concentrations is discussed.



No bacteria or other visible microorganisms have been found upon microscopical examination of the local lesions. Fluids from plants not affected with mosaic do not produce the lesions when used as inoculum. Yet it appeared that formal experiments ought to be carried out with virus suspensions free of bacteria to show that the production of the lesions was not due to the introduction of foreign organisms. Three samples of virus were therefore prepared by adding large numbers of a small bacterium, *Aplanobacter michiganensis*, to virus previously diluted with seven volumes of water. These three samples were then passed through Berkefeld W filter candles. The bacteria were entirely removed from the mixture, as was shown by plating from the filtrates. The virus passed through the filter with no observable decrease in its concentration. The lesions developing on *N. glutinosa* plants inoculated with these sterile filtrates were identical in appearance with those produced by the use of unfiltered samples of virus.

In further support of the view that the virus of mosaic itself is responsible for the production of these local lesions, it may be stated that exactly similar lesions have been produced by the use of a virus sample which had been frozen solid for three years, and by the use of several field samples of virus collected at widely separated points. It is improbable that any second virus or any other organism would be so closely associated with tobacco mosaic virus as to be present in each of these cases.

In order to prove beyond doubt that the lesions are not caused by any foreign organism, they should be produced in a sterile plant by the use of virus freed from bacteria. But the fact that the test plants are grown in sterilized soil from seeds seems sufficient protection against the presence of any organisms on their surface, especially as the individual plants are invariably capable of showing the lesions.

#### Use of local lesions in measuring virus concentrations

It has long been the custom to use the appearance of the systemic disease in *N. tabacum* plants as an indicator of the successful transfer of virus from mosaic plants or extracts from them. Several methods of this kind are in use for the more or less accurate measurement of the relative concentrations of mosaic virus in different samples. In

such work a whole plant is necessary for each successful transfer. As the accuracy of the measurement depends largely upon the number of successful transfers, the numbers of plants required usually prove a limiting factor in the number of measurements which can be made. Obviously it would be a great advantage if many successful transfers could be distinguished on a single plant or even on a single leaf. This advantage is given by the use of the local necrotic lesions here described.

It has been found that the number of successful transmissions of virus can be learned by counting the local necrotic spots at the points of inoculation in such species as *N. rustica* and *N. glutinosa* as well as by using whole plants as indicators. Moreover, since the lesions appear locally before systemic symptoms appear, economy of time is secured.

Since *N. rustica* offers the advantage of very large leaves, a method of using it for measuring virus concentrations will first be described. Dilution curves and a discussion of the accuracy attainable will be given. *N. glutinosa* has proved of great value, because on it necrotic lesions, which can readily be counted, appear very soon after inoculation. The disease may be transmitted by wiping the leaf surface gently with a cloth saturated in virus extract.

In *N. rustica* lesions are most readily counted when produced by pin prick punctures. A set of five insect pins held in a temporary handle has usually been used by the writer for introducing the virus. The pins are alternately dipped in a sample of virus and used to puncture the test plant leaf. In this way large numbers of punctures are rapidly made in a series of leaves. Usually each leaf will accommodate 250 or 500 punctures. The number of necrotic lesions developing is small in comparison with the number of punctures, but is sufficient to allow a fairly accurate reading of virus strength to be made with a few plants. In fig. 1 a leaf inoculated as described is shown. It is necessary to make inoculations of samples on opposite sides of the midvein of the same leaf or to use a large number of leaves for a single test. This is because leaves of different ages have been found to differ somewhat in susceptibility. In general the younger leaves are the more susceptible.

A curve showing the effect of dilution over a considerable range of virus concentrations is presented in fig. 6. The graph is based upon two series of experiments, one with a mosaic virus of usual

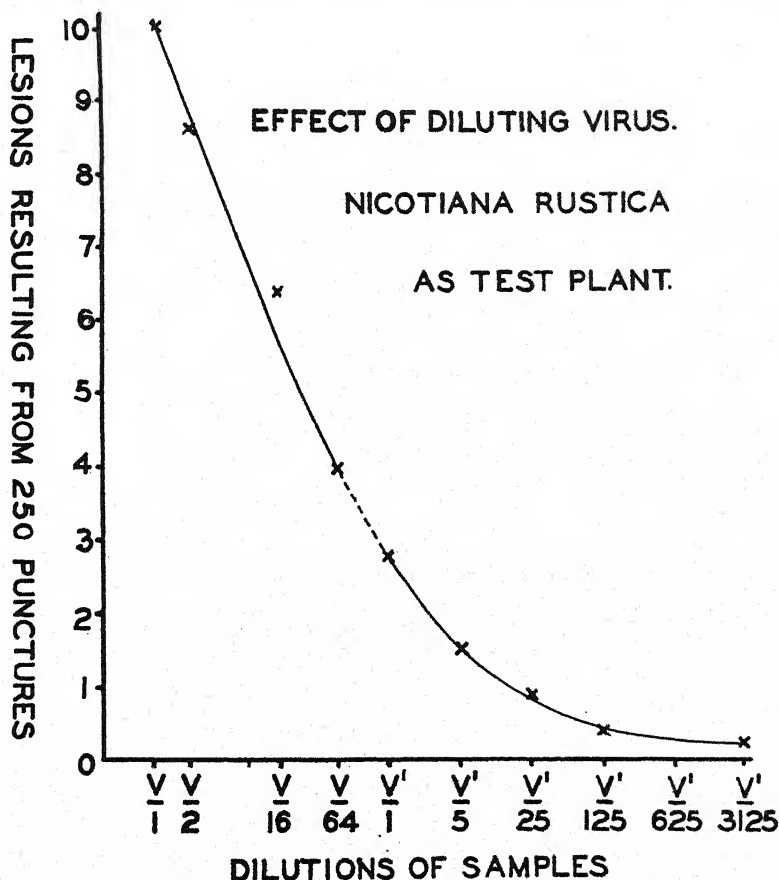


FIG. 6.—Effect of diluting virus when *N. rustica* is used as test plant. Two samples of virus, V and V', used in obtaining the data represented. Virus V was more concentrated than virus V', which was exceptionally weak. The lesions resulting from 38,000 pin prick punctures are represented by the nine points determining the curve.

strength and one with a weak sample. Thirty-eight thousand punctures were made to obtain the transfers represented by the figure. It will be observed that in the region in which four to ten lesions were obtained in each set of 250 punctures, the line appears nearly

straight as drawn to a semilogarithmic scale. This part of the curve is similar to that known from earlier work with commercial tobacco. It is interesting to note the direction of the line when more dilute virus is used. With *N. rustica* it has been possible to study the lower range, because greater numbers of measurements can be made by the use of local lesions than by the use of the systemic disease as an indicator of successful transmission. The upper range is the portion most frequently dealt with, and the range in which the greatest accuracy can be obtained.

#### ***Nicotiana glutinosa* as test plant**

The characteristics of *N. glutinosa* make it a very useful test plant for measuring virus concentrations. It has a low virus content when diseased, which gives it an advantage over *N. tabacum*, in that contaminations do not readily occur when the plants are handled. The rapidity of development of the local lesions makes it possible to have preliminary results of measurements on the second or third day after inoculation, according to the season. On the fourth or fifth day final results may be noted and the plants discarded. Large numbers of lesions can be distinguished on individual leaves; thus a high degree of accuracy may be obtained in comparing virus concentrations. The use of pin punctures is unnecessarily slow in the use of this species as a test plant. In its stead a much more rapid method of inoculation may be used, allowing tobacco mosaic virus to be measured as readily and as rapidly as bacteria are counted by plating methods.

The procedure is as follows. *N. glutinosa* plants are grown in 4-inch clay pots until flower buds begin to appear. At this stage at least five leaves on each plant are of good size. These five leaves are used for the inoculation; and for convenience in manipulation all the remaining leaves of the plant, as well as the growing point, are pinched off. This leaves a sturdy stem supporting five large leaves. Virus from any source to be tested is now taken up on a small piece of white cheesecloth and rubbed once firmly but gently over the entire upper surface of the five leaves. A full stream of tap water is used to wash away excess virus at once after this inoculation. After a little practice the operations become quite uniform. The results

justify the belief that approximate uniformity of inoculation can thus be obtained. Undiluted tobacco mosaic extracts result in the production of about 300–600 lesions on each test plant when applied in this way. If they are diluted with water, the decrease in the number of lesions is at first very rapid, later more gradual. A wide range of concentrations can be studied accurately.

As an illustration of the effectiveness of this method of inoculation, a series of leaves showing the decrease in the number of lesions appearing with decreasing concentrations of virus in the inoculum is shown in fig. 7. A dilution curve showing the effect more accu-

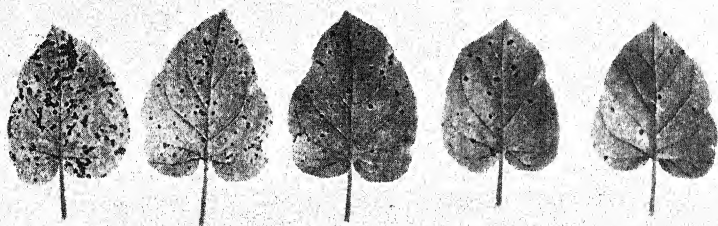


FIG. 7.—Five leaves from plants of *N. glutinosa* used to measure the effect of dilutions (1:1, 1:3.16, 1:10, 1:100, 1:1000). Numbers of lesions do not correspond to the more exact averages shown in dilution graph in fig. 8, but decrease in lesions with serial dilution is plainly shown.

rately is shown in fig. 8. Fig. 9 shows the lower range of this curve more clearly. A curve showing the probable errors of counts of lesions on single test plants is shown in fig. 10. By reference to these curves it will be observed that a very slight dilution, as by a single volume of water, can be detected with satisfactory accuracy by the use of a small number of test plants. In most preliminary measurements a single test plant is sufficient to give an excellent idea of the strength of the virus sample in hand. Even the most important measurements are usually made accurately enough if sixteen test plants are used. Fig. 11 shows two test plants, one five days after inoculation, the other one day after inoculation.

The value of this method of measuring the concentration of tobacco mosaic extracts can best be appreciated when it is compared with the old method for estimating the strength of a sample by

# LESIONS

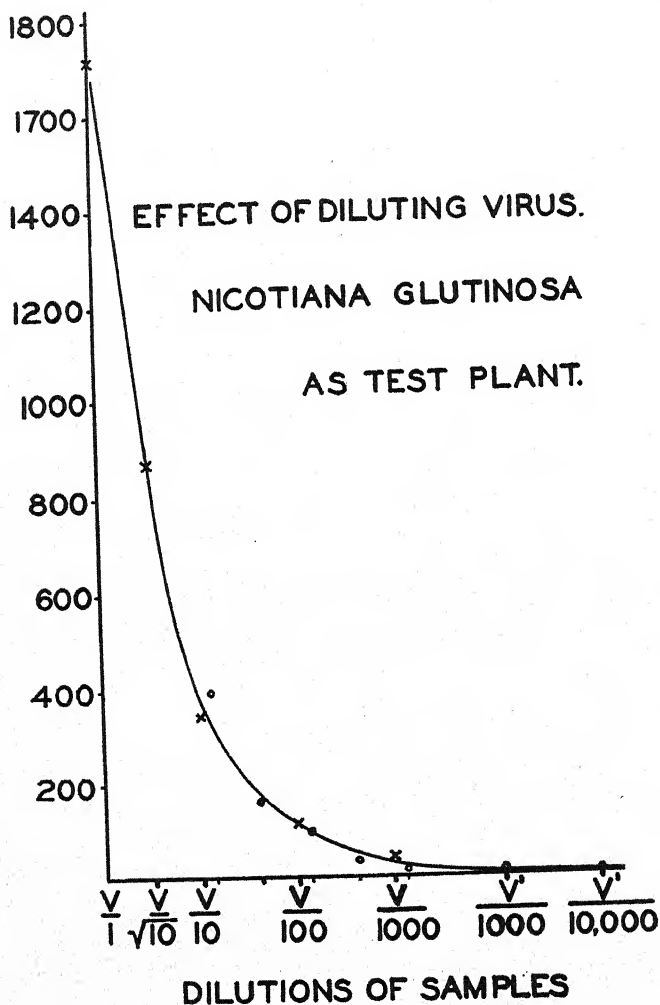


FIG. 8.—Effect of diluting virus sample, *N. glutinosa* as test plant. Counts of lesions in every case represent average number of lesions appearing for each test plant, when five leaves of each plant had been wiped with the virus sample in question. Accuracy attained may be noted by closeness of determined points to the smooth curve. Nine plants used for each determination; probable error of each point as determined is therefore one-third the error shown in fig. 10 for single observations of similar numbers of lesions.

inoculating potted plants of *N. tabacum*. Ten plants of this species were frequently used in a single test. On the average five successful inoculations and five failures would be observed on this number of test plants. On an equal number of *N. glutinosa* test plants in a

## LESIONS

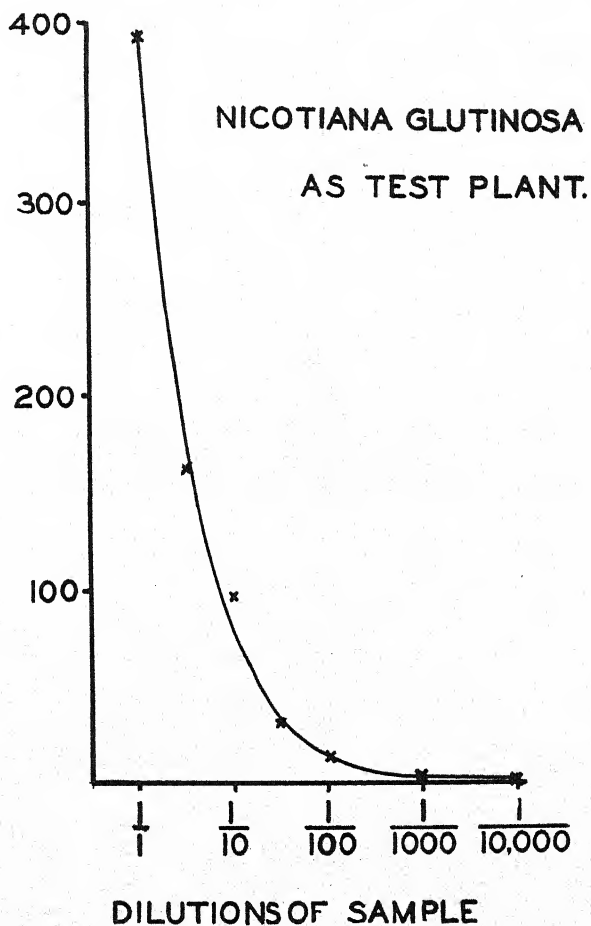


FIG. 9.—More dilute range of curve shown in fig. 8, drawn to such a scale that readings may be made accurately (see fig. 10 for probable errors of single observations in this range of measurements).



typical case 5000 successful inoculations would be observed because of the localized nature of the lesions counted. The accuracy of both

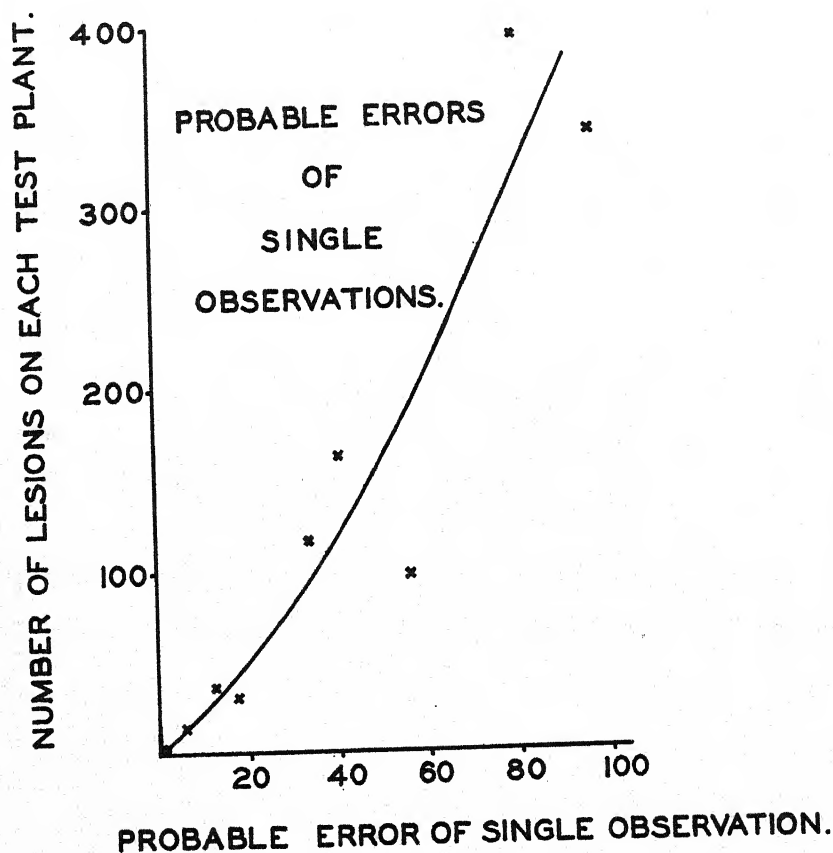


FIG. 10.—Probable errors of single observations for test plants having numbers of lesions between 0 and 400. The error in determining these probable errors is naturally large, and information was insufficient to extend curve beyond 400. At 1714, however, the probable error of a single observation has been found to be 414.

methods depends largely on the number of successful inoculations counted. It is estimated that in the average case for the same accuracy one test plant of *N. glutinosa* used as described serves the purpose for which at least several hundred *N. tabacum* plants are required with the customary methods of using that species.

*N. glutinosa* differs from the other species of *Nicotiana* in two ways. It produces very little virus when successfully inoculated, and symptoms appear on the upper parts of the plant to a very limited extent. Measurements of the amounts of virus produced by this plant have shown that the concentration present does not exceed the strength of an ordinary sample of commercial tobacco mosaic virus diluted with water to one-five hundredths of its original strength. The systemic course of the disease is not like that of most

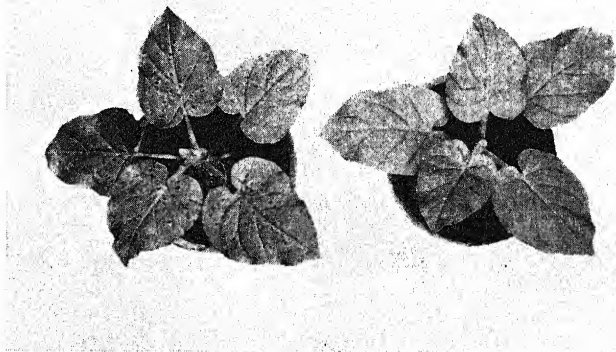


FIG. 11.—Test plant of *N. glutinosa* (at left) as used to determine virus concentrations, inoculated five days before it was photographed. Similar plant (at right) inoculated one day before it was photographed. Lesions had not yet appeared on this recently inoculated plant.

species. Generally the growing tip soon shows the same type of symptoms which have occurred where the virus was introduced. The writer has never seen a case in which the developing leaves of *N. glutinosa* were affected with necrotic spots. The local lesions enlarge day by day, laying down ring after ring of dead tissue. After a week or so new secondary spots are sometimes formed on the peripheries of the extended primary lesions. Later lesions may appear on leaves younger than those inoculated, but they do not affect the developing leaves. Sometimes veins and portions of the stem are killed by the extending lesions. The green portions of the leaves between the primary lesions are not suitable sources of virus. No successful trans-

fers have been secured when juice from them has been used as inoculum. The virus seems to be confined to the visible lesions.

*N. glutinosa*, like *N. rustica*, shows a gradient of susceptibility when successively older leaves are considered. The young leaves tend to produce more local lesions when inoculated with the same source of virus as neighboring older leaves. This condition is partially remedied by removing the growing point of the plant, as is done in using the plant for measurements. In two series of plants, one with the growing tops attached, the other with the tops removed, this condition of affairs was demonstrated. In the first series, in which the growing points remained attached, the totals from top leaf to lowest leaf were 6066, 5117, 4292, 3222, and 3198. The gradient is very marked. In the second series, in which the growing points were removed, the totals were 4795, 4778, 4227, 4320, and 4343. The gradient is much less. The practice of removing the tops insures less error in comparing leaves of different age, since the chance choice of an older or a younger leaf in any plant will introduce less variation when the difference in the susceptibility is decreased.

### Discussion

The recognition of symptoms which appear at the site of inoculation opens a new field of investigation in connection with tobacco mosaic. The first development of the disease seems to be very strictly localized. It is only the later course of the infection which is marked by changes in appearance of the developing leaves. The species of *Nicotiana* differ markedly in the conspicuousness of their local symptoms. In some cases necrotic spots are formed, in others a pale yellow area marks the point of entrance and early development of the virus.

Most of what is known regarding virus diseases of plants has been learned from inoculation studies. The behavior of the virus under experimental conditions, such as filtration, purification by chemical processes, and contact with disinfectants has always been judged by its reinoculation into living plants. Such tests have been difficult to carry on with a high degree of accuracy, because of the large numbers of plants required for each determination. The use of local necrotic lesions makes it possible to recognize very large num-

bers of successful transmissions on single plants. This reduces the amount of labor in many experiments. A degree of accuracy never before possible can be attained in this way with the use of moderate numbers of plants. *N. glutinosa* lends itself particularly to use in measuring virus concentrations, since the necrotic lesions in this plant develop in from two to five days. These lesions are easily counted and their relative numbers show variations in the concentrations of samples with great clearness.

When very small numbers of lesions develop on *N. glutinosa* because of the use of highly diluted samples of virus, there occur many cases in which a plant shows a single local lesion. Under such circumstances it seems possible that the infection has resulted from the entrance of a single virus particle. Transfers from this type of plant may be assumed to develop virus produced from this single particle. The process of isolation may be repeated if desired. Possibly a pure line of the causal agent is thus secured. Such single particle strains have not yet been carefully studied; their isolation and use may be expected to furnish material for future work. The use of *Nicotiana* species which show local necrotic lesions is as helpful in the study of tobacco mosaic virus as KOCH's plate method is in the study of bacterial cultures.

### Summary

1. Five *Nicotiana* species were found to develop necrotic lesions wherever virus of the common field type of tobacco mosaic successfully entered leaf tissues. These species were *N. rustica*, *N. langsdorffii*, *N. sanderae*, *N. acuminata*, and *N. glutinosa*.

2. The local lesions developing in *N. rustica* can be used to measure accurately the potency of tobacco mosaic virus.

3. The local lesions in *N. glutinosa* are exceptional in the rapidity of their development. They sometimes begin to appear thirty hours after inoculation. In four or five days they are well developed. Large numbers of them may be distinguished on a single plant. This allows comparisons to be made between virus samples, since the number of lesions developing depends on the virus concentration of the inoculum.

4. A standardized method for using *N. glutinosa* as a test plant for measuring the concentration of mosaic virus gives as rapid and as

accurate results as the determination of bacterial numbers by plating methods.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.  
YONKERS, N.Y.

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## INOCULATING METHODS IN TOBACCO MOSAIC STUDIES<sup>1</sup>

FRANCIS O. HOLMES

(WITH FOUR FIGURES)

The virus of tobacco and tomato mosaic disease has usually been transferred from plant to plant in mosaic studies by scratching or pricking leaves, and wetting the injured areas with extracts from mosaic plants. As a rule the virus is gently rubbed into the wounds. This process successfully transfers the infection and has been accepted widely. Occasional failures to secure the expected results suggested the advisability of making an analysis of inoculation methods. The purpose of the present study was to ascertain which methods of introducing virus were most effective.

At the beginning of the work, the problem in hand was the simple question of the proper depth of scratch to make in the surface of a leaf before wetting the area with virus. The method used was that described in the preceding paper.<sup>2</sup> *Nicotiana rustica* plants were inoculated in the ways which were to be tested, and the numbers of lesions resulting were used as indications of the numbers of successful transfers. This process soon showed that different types of wounds were very different in effectiveness.

To determine whether shallow or deep scratches were the more effective in aiding the virus to enter the tissues, the following experiment was performed. Several series of scratches, some shallow and some deep, were made on individual leaves of the test plants. Virus was applied at once by wetting a small piece of cheesecloth and lightly wiping the surface of the scratched leaf. It was expected at the time that the virus would enter some types of wound more readily than others, and that numerous necrotic lesions would cluster around the scratch of most favorable depth. If this had been the

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

<sup>2</sup> HOLMES, F. O., Local lesions in tobacco mosaic. BOT. GAZ. 87: 39-55, 1929.

case, it would have been clear whether broken surface cells or deeper layers were the more receptive. When the lesions appeared, however, it was found that very few of them were on or near the scratches. They were well distributed over the general surface of the leaf between and around the scratches. A typical leaf from such an experiment is shown in fig. 1. Apparently the scratching had been an unimportant part of the inoculation method. The light rub-

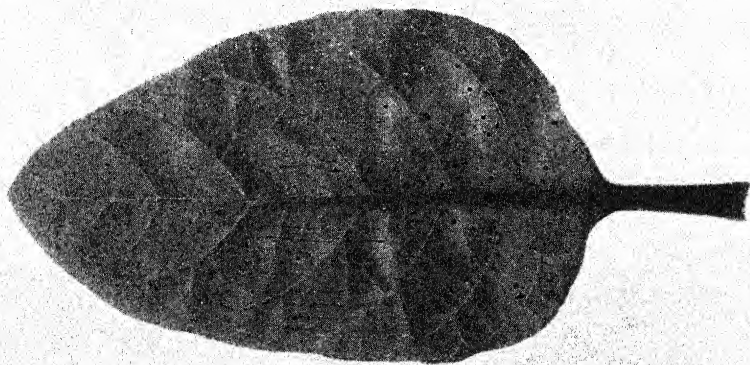


FIG. 1.—Leaf of *Nicotiana rustica* on which 20 scratches were made with needles, and virus was rubbed over the scratches and whole surface of the leaf. Resulting lesions were photographed when small, and appear as tiny dark spots, very few of which are on or near the scratches. On original leaf at time of photographing the lesions were brown, the scratches green like the general leaf surface.

bing must have produced most of the wounds necessary for the entrance of the virus.

To secure further evidence on this point another experiment was carried out. The scratching and rubbing operations were separated. On one side of the midvein of a leaf of a healthy test plant ten scratches were made. On these an extract from mosaic plants was dropped and spread with care to avoid injuring the surface near the wounded area. In the same way 200 scratches were made on twenty leaves. On the other side of the midveins the same virus was rubbed lightly into small areas of the leaves. No scratches were made here, nor was any distinct injury to be seen after the application of the virus. On a given leaf of *Nicotiana rustica* the tissues



on one side of the midvein of a leaf are precisely as susceptible as the tissues on the other side of the midvein. If the two methods of inoculation were equally effective, equal numbers of local lesions would be expected on each side of the leaves treated. When the lesions appeared, however, it was found that they were numerous on the side of each leaf into which virus had been rubbed; but they were rare or absent on the side which had been scratched and then wet with a liberal amount of the mosaic extract. On the 200 scratches only four lesions developed. Two leaves from a series of such experiments are shown in fig. 2.

A similar test was carried out to discover whether scratching through a drop of mosaic extract was effective. Twenty-five separate leaves were scratched, ten times each, through drops of the fluid. On these leaves the same virus was rubbed into similar small areas. Great numbers of lesions appeared from the rubbing, but only 13 lesions were found on the 250 needle scratches. Scratching through mosaic extract, therefore, appears slightly more effective than dropping virus on scratches, but neither method proved to be efficient as compared with light rubbing. These experiments make it seem probable that when scratching and rubbing methods of inoculation are used, the scratches are relatively ineffective, and the rubbing constitutes the effective portion of the process.

It was not possible to obtain so clear a demonstration of what happened when similar experiments were performed with commercial tobacco, because of the difficulty in detecting the spot through which infection took place. Nevertheless a test was made to see whether the response was similar. Forty-four plants of Turkish tobacco were inoculated by making ten scratches on each and immediately wetting these wounds with mosaic extract. Forty-four additional plants of the same lot were inoculated with the same mosaic extract by the simple process of rubbing lightly over a small area with a piece of cheesecloth, wetted in the same source of virus. When the plants began to show symptoms, it was found that only eight successful inoculations in forty-four trials had occurred in the group inoculated by scratching and wetting the scratches with virus. On the other hand, forty successful inoculations had resulted from the forty-four trials in the group subjected to light rubbing with

cheesecloth wet with mosaic extract. This experiment shows that *Nicotiana tabacum* behaves like *N. rustica*, in that it is not readily

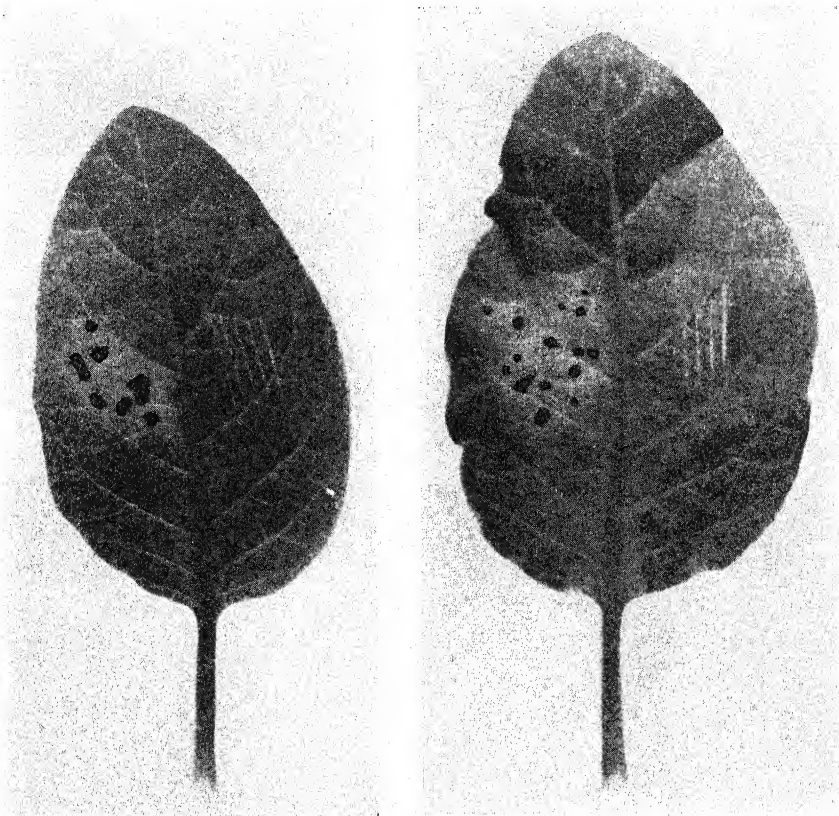


FIG. 2.—Two leaves of *Nicotiana rustica* typical of a large series in which scratches were made on one-half of each leaf and immediately wet with virus extract from a dropper; on remaining half a small area was rubbed gently with cheesecloth saturated with the same virus used to wet the scratches. Rubbing proved more effective than scratching as an inoculating method.

inoculated through scratches wetted with virus, but is more easily inoculated by light rubbing.

It seemed desirable to know whether wounds made by rubbing would be more effective when made in the presence of the virus, or whether they could be made and subsequently inoculated with equally good results. To test this a comparison was obtained in

the following way. A piece of dry cheesecloth was used to rub one-half of a leaf. Mosaic juice was then dropped at once upon this surface. The same kind of cloth wetted with the same source of virus was then used to rub the opposite half of the leaf. Lesions developed in abundance on leaf areas rubbed with cloth wet with virus, but areas rubbed first with a dry cloth and then wetted with virus developed very few necrotic spots. This is illustrated by the leaf shown in fig. 3. It was found that if a water-soaked cloth was used to break the hairs instead of a dry cloth, the result of the experiment was the

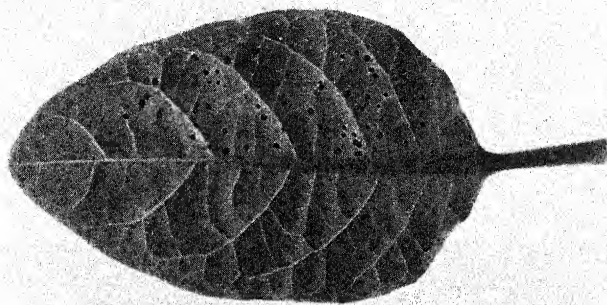


FIG. 3.—Leaf of *Nicotiana rustica* on one-half of which virus was dropped after rubbing with cheesecloth had broken the hair cells (one lesion appears); on the other half the rubbing was with cheesecloth saturated with sample of juice used on the other half. Rubbing is effective as an inoculation method only in presence of the virus.

same. The virus did not enter the wounds made before it was applied, but required wounds made in its presence.

If virus cannot readily enter wounds a few seconds after they are made, it must be that the actual entrance is completed almost instantaneously; otherwise wounds would quickly become useless although made in its presence. If the virus enters instantaneously, the practice of renewing mosaic extract above wounds and keeping it there for long periods to allow extended opportunity for entrance may be useless. To test whether it is or not the following experiment was performed. On one side of a leaf the hairs were broken with a cloth wet with water, and the area then flooded with fresh extract from a mosaic plant. On the other half of the leaf the same

kind of cloth was used, after wetting it with the same juice, to rub lightly the remaining leaf surface. As soon as the process was complete a full stream of water was used to wash the virus from this second half of the leaf, but virus was renewed over the water-rubbed portion so as to keep it wet for several minutes. Thus the first portion of the leaf was apparently favored by long contact with the virus; yet an overwhelming majority of the lesions appeared on the portion rubbed for a moment with virus and then washed clean with a stream of water. Many repetitions of this process showed the same result.

This experiment supplied additional evidence that wounds made immediately before the application of virus were ineffective. It also indicated that when virus was applied directly, it was taken up so quickly that the excess might be washed away at once without making the inoculation ineffective.

To test the possibility that the washing away of the excess virus might decrease the count to a slight extent, a quantitative experiment was performed. A group of plants were inoculated by rubbing the whole leaf surfaces with mosaic juice. As soon as each leaf was treated, one-half was washed with a stream of water. When the lesions appeared they were carefully counted for the washed and unwashed halves of the leaves. Normally the two halves of the leaves would show approximately equal counts and there would be a chance variation from side to side, but under these two treatments there was a definite response in favor of the washed portions. On the washed halves there were 1221 lesions, and on the unwashed halves 964. Nine of the twelve leaves counted showed excess lesions on the washed portions. The exceptions were nearly equal counts on three leaves. It was further noted that on eleven of these twelve leaves the lesions were slightly earlier in appearing on the washed portion. This observation lends further support to the view that the washing favored the inoculation and did not hinder it. Perhaps the water hastened the recovery of some cells by washing away the excess of toxic materials.

These experiments give some insight into what happens when virus is applied to susceptible plants. Small wounds, imperceptible to the unaided eye, take up virus instantly. Wounds made before

virus is applied are nearly or quite useless. Excess of virus may be removed at once with a stream of water without decreasing the number of effective invasions of the plant by the virus.

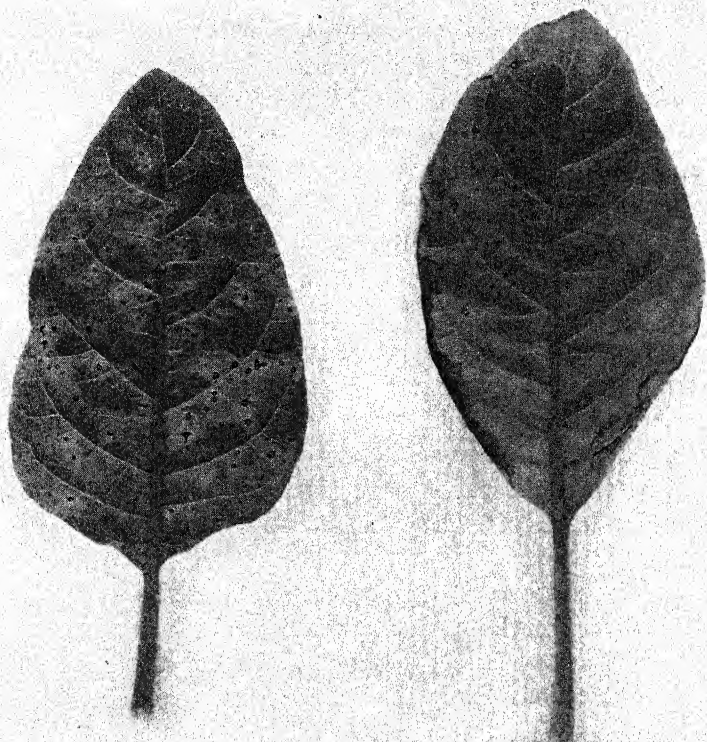


FIG. 4.—Two leaves of *Nicotiana rustica*, each of which was rubbed over its whole upper surface with cheesecloth saturated with the same sample of virus (preserved in 50 per cent glycerin). Mixture was allowed to stand on surface of leaf at right. Injury from presence of glycerin appears on edges of leaf and few lesions are in evidence. Leaf at left was washed with full stream of water at once after treatment. No injury noticeable in such cases, and abundance of lesions develop.

Practical use of this information can be made. It is often desired to treat virus with chemicals harmful to plant tissues. The virus may survive the treatment, but tests of the resulting mixture may be difficult because of the killing of the leaves by the chemicals



present. If the mixture is applied by rubbing and is then washed off with water to remove the excess, successful inoculations may be obtained.

Fig. 4 shows at the right a leaf rubbed with virus in 50 per cent glycerin. The presence of the glycerin soon caused drying of portions of the leaf and the death of certain areas. A similar leaf treated with the same glycerinated virus is shown at the left. It was washed with water at once after the inoculation. The lesions developed very much more freely in this second case, and the leaf itself showed no injury from its brief contact with the chemical. It seems probable that immediate washing of inoculated leaves when any foreign materials are applied with the virus will allow more successful transfers to be made.

### Summary

1. The most effective way of transferring mosaic virus to tobacco plants has been found to be gentle rubbing over a large leaf surface with a cloth soaked in extract from mosaic plants. Scratches are much less effective, even when made in the presence of the virus.

2. The virus of tobacco mosaic does not readily enter wounds made in the leaves of healthy tobacco plants if these are made before its application.

3. Entrance seems to be instantaneous upon the production of a suitable wound in the presence of the virus. Immediate removal of the excess of mosaic extract by washing does not decrease the total number of infections, but in some cases actually increases it.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.  
YONKERS, N.Y.

## MICROCHEMICAL STUDIES OF ROOTING AND NON-ROOTING ROSE CUTTINGS<sup>1</sup>

MARGERY C. CARLSON

(WITH SIX FIGURES)

### Introduction

Cuttings of overwintered canes of Dorothy Perkins rose produce shoots which give rise to roots when placed in humid air or in a moist medium (fig. 3). American pillar rose, treated in the same way, produces shoots but no roots, as shown by ZIMMERMAN (14). It seemed that a comparison of the anatomy and composition of such closely related plants with so marked a difference in response might give some idea of the factors influencing their rooting behavior.

Dorothy Perkins and American pillar are hybrids with a common parent. Dorothy Perkins results from a cross of *Rosa wichuraiana* × Mme. Gabriel Luizet, and American pillar from a cross of *R. wichuraiana* × *R. setigera* (1). They are climbing roses of the *multiflora* type, producing long, unbranched canes one season and flowers the second season. Figs. 1 and 2 show the vegetative canes of each rose. Dorothy Perkins has longer and more flexible canes, smaller leaves, and smaller thorns than American pillar.

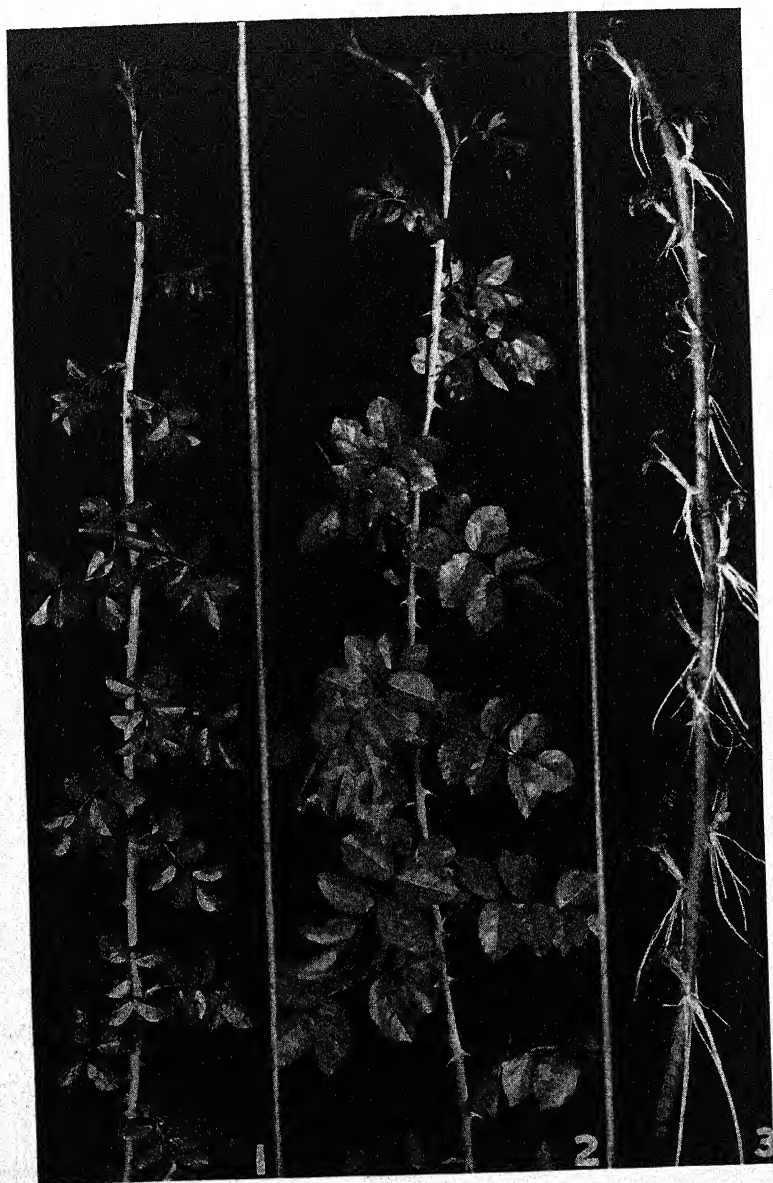
### Material and methods

Canes were cut into pieces 10-15 inches long, and placed with their basal ends in water in saturated atmosphere, either in a large humidity case in the greenhouse, or under bell jars in the laboratory. The humidity case was aerated only by the frequent opening of the door, but air was drawn through the bell jars by means of a filter pump.

Microchemical examinations of transverse and longitudinal sections of nodes and internodes were made at the time of collection,

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FIGS. 1-3.—Figs. 1, 2, vegetative canes of Dorothy Perkins (left) and American pillar (right); fig. 3, cutting of Dorothy Perkins, showing young branches and roots produced in saturated atmosphere.

and at intervals of two to four days throughout the experiments. Six series of examinations were made as follows:

SERIES NUMBER	DATE STARTED	DURATION OF EXPERIMENT (DAYS)
1.....	March 15, 1926	31
2.....	March 24, 1926	28
3.....	April 7, 1926	16
4.....	April 22, 1926	16
5.....	March 6, 1927	18
6.....	April 6, 1927	19

The microchemical tests described by ECKERSON (2) were used.

STARCH.—Iodine solution in potassium iodide.

FRUCTOSE.—(1) Copper tartrate and 20 per cent sodium hydroxide, cold; (2) phenylhydrazine-hydrochloride and sodium acetate, 12 hours at room temperature.

GLUCOSE.—(1) Copper tartrate and 20 per cent sodium hydroxide, heated 1–2 minutes at 40° C.; (2) phenylhydrazine-hydrochloride and sodium acetate, heated 24–48 hours at 40° C.

OTHER REDUCING SUBSTANCES.—Copper tartrate and 20 per cent sodium hydroxide, heated 15–20 minutes at 40° C.

PROTEIN.—(1) Iodine solution in potassium iodide; (2) Biuret reaction: 5 per cent copper sulphate and concentrated potassium hydroxide.

ASPARAGIN.—Absolute alcohol on sections and identification of crystals which precipitate out.

NITRATES.—Diphenylamine in 75 per cent sulphuric acid.

CALCIUM.—(1) Five per cent sulphuric acid; (2) identification of calcium oxalate crystals.

POTASSIUM.—Platinum chloride.

MAGNESIUM.—Ammonium chloride, ammonia, and sodium phosphate.

PHOSPHORUS.—Magnesium sulphate and ammonium chloride.

TANNIN.—Ten per cent ferric chloride.

### Results

OVERWINTERED CANES.—In March and April, Dorothy Perkins canes brought in from the garden contained much more reserve starch than American pillar canes. The starch was most abundant

at the nodes and decreased slightly from the base of the canes upward. In those of Dorothy Perkins the cells of the primary and secondary medullary rays, of the primary xylem parenchyma, and of the outer pith and bud gap were filled with starch. The small active cells of the inner pith were filled with starch at the nodes and partially filled in the internodes. The endodermis sometimes con-

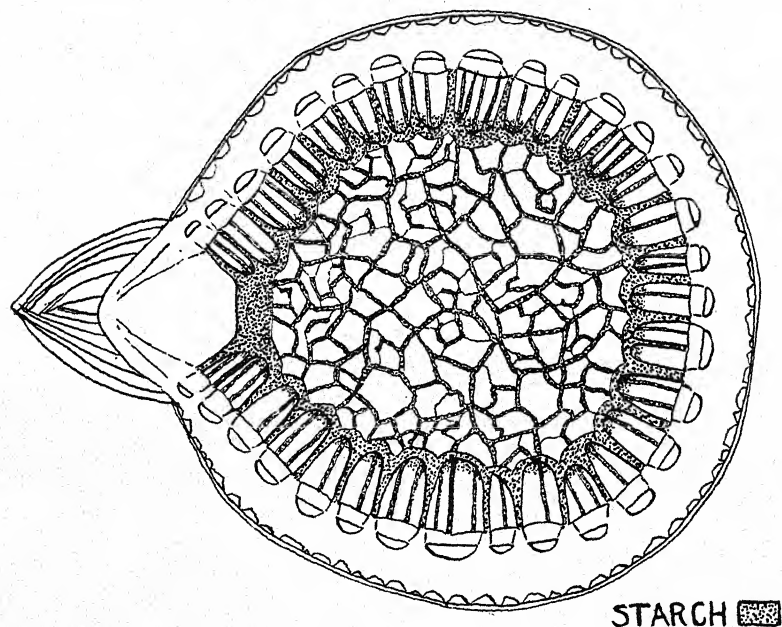


FIG. 4.—Diagram of transverse section of stem of Dorothy Perkins rose, showing arrangement of tissues and location of starch.

tained starch (fig. 4). In American pillar stems some of the medullary ray, outer pith, and bud gap cells were partially filled with starch, and the small cells of the inner pith contained a few grains each at the nodes, but were free from starch in the internodes.

There was no fructose and very little glucose in either rose. Other reducing substances (dextrin-like substances and tannins possibly) were usually present in abundance in both roses. The pith and cortex of the bud contained more of these substances than the pith and cortex of the cane. The pith of the cane sometimes con-

tained more near the bud than on the side opposite the bud. The embryonic region, leaf primordia, and procambial strands of the bud were usually free from reducing substances.

Those proteins which give the biuret reaction were present in the meristematic tissues of the bud and in the cambium and inner phloem of the cane. The cells of these tissues were filled with cytoplasm, and contained very large nuclei with large nucleoli. The pith and cortical cells of the bud also contained much protoplasm. The cells of the cambium and phloem parenchyma of the cane were high in content of protoplasm; the cells of the cortical parenchyma were lined with cytoplasm containing chloroplasts; while the small pith cells, medullary ray, and bud gap cells contained little protoplasm. The large pith cells were empty.

Tannins occurred very abundantly in the epidermis, sclerenchyma, scattered cells of the cortical parenchyma, endodermis, and in some of the cells of the bud gap and outer and inner pith.

Sphaero-crystals of calcium oxalate were more abundant in American pillar than in Dorothy Perkins. They were present in largest amounts in the pith of the bud and in the bud scales. The cortex and pith of the cane contained a considerable amount. Single crystals, probably also mostly calcium oxalate, were present in rows of cells in the sclerenchyma, collenchyma, and phloem of the cane.

The canes collected March 15, 1926, were made into cuttings and placed in water in saturated air (series 1). The changes in the content of starch and reducing substances in this series are given in table I.

All buds along the canes, except those under water, developed rapidly. As the buds grew, the reserve starch disappeared from the canes in the following order: (1) from the small cells of the pith in the internodes; (2) from the small cells of the pith at the nodes, beginning on the side opposite the bud; (3) from the outer pith and primary xylem parenchyma, beginning opposite the bud; (4) from the medullary rays, beginning opposite the bud; (5) from the bud gap.

After a few days in saturated air, a swelling appeared on the lower side at the base of the new shoots produced by cuttings of Dorothy Perkins. It usually continued to develop around the base,

TABLE 1  
FERTILIZING SUBSTANCES IN CUTTINGS OF DOROTHY PERKINS AND AMERICAN PILLAR ROSES; SERIES I, 1926

CHANGES IN STARCH AND REDUCING SUBSTANCES IN CUTTINGS OF DOROTHY PERKINS AND AMERICAN PILLAR										
DATE	CONDITION OF MATERIAL		STARCH		FRUCTOSE		GLUCOSE		TOTAL REDUCING SUBSTANCES	
	Dorothy Perkins	American pillar	Dorothy Perkins	American pillar	D.P.	A.P.	Dorothy Perkins	American pillar	Dorothy Perkins	American pillar
March 15 . . . . .	B 2-3 mm. dormant	B 4-5 mm. dormant	MR+++++ BG+++++ P+++++	MR+++ PN+++ PI*	N* I*	N*	N++ I+	N+ I+	N+++++ I+++++	N+++++ L+++++
March 18 . . . . .	B 8-9 mm.	B 7-8 mm.	MR+++++ BG+++++ P+++++	MR+++ PN+++ PI*	N*	N*	N++++	N+++++	B+++++	N+++++
March 20 . . . . .			MR+++++ BG+++++ OP+	MR+++ PN+++ PI*	N*	N*	N+++++ B(near B) P+++++	N+	B+++++	B+++++
March 22 . . . . .	Br 16 mm.	Br 10-15 mm.	MRN++++ PN+++++	MR+ (near Br) P*					N+++++ Br+++++	N+++++ L+++++
March 26 . . . . .	R just visible	R none	MR+++++ P* (near Br)	MR+ (near Br) P*	N+	N+	PN++++ PI+++	PN+++ PI+++	N+++++ Br+++++	N+++++ Br+++++
March 29 . . . . .	Br 18 mm. L 4	Br 10-15 mm. L 3	MR+++++ (near Br)	MR+ (near Br)	N*	N*	P++++	P+	N+++++	N+++++
April 1 . . . . .	Br 30 mm. S 3 mm. R 6-8 mm.		MR+++ OP+++ BG+++ (near Br)	MR* BG+	N*	N*	P+++++	P+	N+++++	N+++++
April 6 . . . . .			MR+++ P* BG+	MR* BG* N*	N*	N*	Br+++++		N+++++	N+++++
April 8 . . . . .	R 10 mm.	L 5 R none	MR+++ BG+++ OP+	MR* BG* N*					N+++++ Br+++++	N+++++ Br+++++
April 15 . . . . .	Br 25-35 mm. R 10 mm.	Br 30 mm. L 4 R none	BR+++++ BG+++++	BR* BG* N*	N+	N+	N++	N+	Br+++++ N+++++	Br+++++ N+++++

R to mm.	R none	BG++
<p>Letters show position on stem of materials tested: B, bud; BG, bud gap; Br, branch; I, internode; IP, inner pith; L, leaf; MR, medullary ray; OP, outer pith; P, pith; N, node; R, root. The symbol (*) and plus marks (+) show amount of storage material present: *, trace to none; +, very little; ++, little; +++ moderate amount; ++++, considerable; +++++, excessive amount.</p>		

forming a collar-like enlargement (fig. 5). Roots appeared from this enlargement after eleven days in saturated air. At this time no enlargement and no root primordia had appeared in the young shoots of American pillar cuttings.

At the time roots appeared, Dorothy Perkins canes still had much starch, only that in the cells of the inner and outer pith having

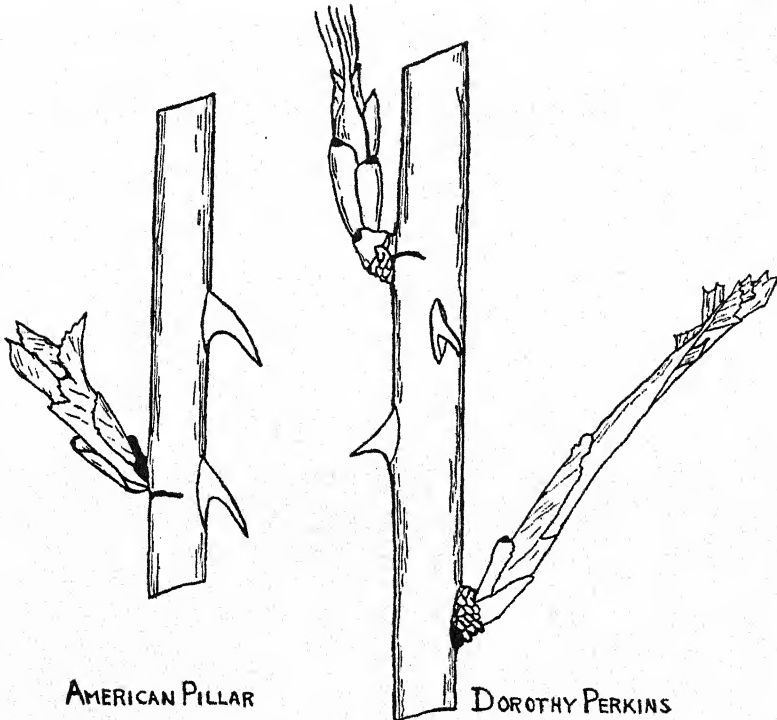


FIG. 5.—Cuttings of American pillar and Dorothy Perkins roses after 8–10 days in saturated air; roots appearing from the swelling at bases of shoots of Dorothy Perkins.

been hydrolyzed; while American pillar had lost all but a few grains in a few cells of the medullary rays, outer pith, and bud gap.

By the end of the experiment the new shoots of Dorothy Perkins were 2.5–3 cm. long, and the roots were 7–10 mm. long; while shoots of American pillar were 3 cm. long and had no roots. Dorothy Perkins still had starch in cells of the medullary rays, outer pith near



the branch, and in the bud gap; whereas all starch had disappeared from the tissues of American pillar.

No fructose was found in either rose at the beginning of the experiment. The glucose content varied in different canes, but there was usually a small amount present. After being placed in humid air the amount of glucose increased in Dorothy Perkins cuttings, and remained high until near the end of the experiment, when it dropped somewhat. Glucose was usually most abundant in the pith of the cane and young shoot. It was sometimes present in the cortex of the cane, with generally more on the side near the branch than on the opposite side. Often there was a considerable amount in the vascular cylinder, especially in the xylem vessels of the young shoot, but practically none was found in the meristematic tissues.

In American pillar cuttings the amount of glucose increased during the first few days in moist air, then decreased and remained low throughout the rest of the experiment. Glucose was high while the starch content was high, and dropped off when the starch content became low.

Reducing substances, not sugars, were present in large amounts in both roses throughout the experiment. Dorothy Perkins sometimes had more than American pillar. These substances were found chiefly in the pith of the branch and cane and in the vascular tissue.

These statements regarding reducing substances are based on the time required for reduction of copper tartrate. The osazone test, which was always made at the same time, was consistently negative for reducing sugars. This may have been due to the fact that the concentration of sugars was too low for osazone crystal formation, or to the fact that the presence of some substance (or substances) prevented the reaction. Later in the year, when the sugar content of the canes from the garden was much higher, the osazone test was positive.

The tests for nitrates were negative for both roses throughout the experiment.

Potassium was always abundant in all tissues of both roses, especially in the phloem, in the meristematic tissues, and in the pith of the branch.

The amount of calcium oxalate did not change noticeably in



either rose during the experiment. When the buds grew, the large sphaero-crystals of calcium oxalate in the pith remained at the very base of the young branches. Soluble calcium compounds were abundant in the young branches of both roses.

Proteins increased in the developing branches, but no decrease in protein content was detected in the canes. The enlargement at the base of young branches of Dorothy Perkins cuttings was due to an extraordinary development of phloem parenchyma. This tissue was high in protein content. The root primordia were first detected in this new tissue as small regions of cells which gave a pinkish color with the biuret reaction.

When kept for a few days in saturated air a dense precipitate formed with absolute alcohol in certain cells of the pith and cortex of the new shoots and in the small cells of the pith of the cane. This precipitate was not identified. After 10 or 11 days asparagin appeared in great abundance in both roses in those cells which had formerly contained the substance precipitated by alcohol. Asparagin remained high until the end of the experiments.

All of the later series gave results similar to those of series 1. In the spring of 1927, however, the difference in the starch content of the two roses was less striking than in 1926, but Dorothy Perkins again contained more than American pillar.

Fig. 6 shows the comparative changes in the starch content of the two roses in series 6. It will be seen that Dorothy Perkins canes contained considerably more starch than American pillar at the beginning of the experiment. Starch began to decrease in the canes of both roses when they were placed in humid air. In ten days roots appeared at the bases of the new shoots of Dorothy Perkins, but no roots developed on American pillar branches. On April 16, when shoots of Dorothy Perkins cuttings rooted, the canes still contained much starch at the nodes, while American pillar canes contained only a trace. At the end of the experiment, Dorothy Perkins still had a little starch, but American pillar had none.

In series 3, cuttings of both roses were placed under bell jars, some of which were covered with black paper. The temperatures in the bell jars, covered and uncovered, were the same at a given time, but varied with the room temperatures. Practically every new shoot

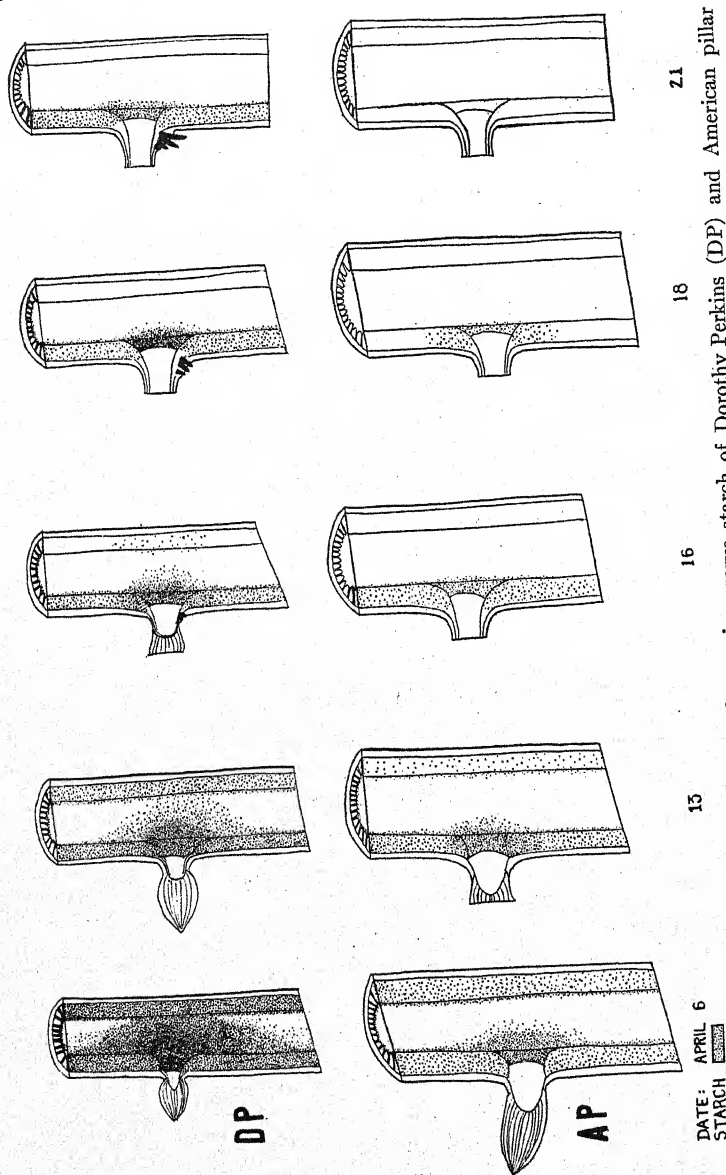


FIG. 6.—Diagrams showing comparative changes in reserve starch of Dorothy Perkins (DP) and American pillar (Ap) cuttings during series 6; roots visible in Dorothy Perkins cuttings on April 16.

of Dorothy Perkins rooted in nine days, in light and in darkness; while no American pillar shoots had rooted in 18 days, when the experiment was discontinued. The changes in reserves were the same in darkness as in light.

In the spring the rooting behavior was the same, whether canes were cut into pieces or left on the plant. Canes attached to the plants were placed parallel to each other on the ground and layered with moist peat moss for a distance of about 20 inches in the middle region. The buds, which were only slightly swollen on March 28, when the experiment was started, developed normally into flower branches. On June 24 each new branch of Dorothy Perkins had a good root system at its base, while no American pillar branches produced roots.

**SUMMER CANES.**—The composition and rooting behavior of new canes of both roses were studied during one season. During the summer the canes elongated very rapidly. Starch was present only in the endodermis, reducing substances were high, no nitrates were detected, and calcium oxalate crystals appeared early in the pith and scales of the newly formed buds. No marked differences in the composition of the two roses were noted during their early period of growth.

American pillar canes ceased their rapid growth in September, but Dorothy Perkins canes continued to elongate until November. Starch began to accumulate in the cells of the medullary rays and outer and inner pith as growth became slower. Deposition proceeded from the base of the canes upward. The starch content of American pillar canes reached its maximum during December and then began to decrease. The maximum starch accumulation in Dorothy Perkins occurred in January and February.

Reducing sugars were more abundant in the canes while starch was accumulating than at any other time. The osazone test showed that there was an abundance of fructose and glucose in the pith, xylem, and phloem, with more at the nodes than in the internodes. After the maximum in starch accumulation had been reached, the reducing sugars were low.

New canes were made into cuttings at intervals during the summer and fall, and were treated in the same way as the overwintered

canes. The upper one or two buds of each cutting developed into branches, but the other buds remained dormant. Cuttings of both roses taken in July and August rooted from the bases of the cuttings, but not from the bases of the new shoots. As already stated, the starch content of both roses was very low during this time. Cuttings taken in the fall, when starch was accumulating in the canes, rooted from the bases of the developing branches and not from the canes. Four of eight branches of American pillar cuttings rooted, and all of eight branches of Dorothy Perkins cuttings rooted. American pillar branches produced one to four roots each, while Dorothy Perkins branches produced seven to twelve roots each. In all cases the rooting required two months for American pillar and one month for Dorothy Perkins. Unfortunately the number of cuttings used in this experiment was too small to make the results conclusive.

Experiments were performed between December and March to determine the effect of temperature on the rooting of cuttings in saturated air. Cuttings consisting of two or three nodes each were placed in constant temperature ovens at  $32^{\circ}$ ,  $25^{\circ}$ ,  $20^{\circ}$ ,  $15^{\circ}$ ,  $10^{\circ}$ , and  $5^{\circ}$  C. for one month. The results are shown in table II. As reported by ZIMMERMAN (14), rooting occurred throughout a wide range of temperature, and the time required varied with the temperature. Dorothy Perkins rooted well from the bases of young branches at  $25^{\circ}$ ,  $20^{\circ}$ , and  $15^{\circ}$ , more slowly at  $10^{\circ}$ , and poorly at  $32^{\circ}$  and  $5^{\circ}$ . Rooting was equally good on pieces from bases, middles, and tops of canes. A few American pillar cuttings taken from the bases and middles of old canes rooted from the bases of young branches at  $25^{\circ}$ ,  $20^{\circ}$ , and  $15^{\circ}$  C.; none rooted at  $32^{\circ}$ ,  $10^{\circ}$ , or  $5^{\circ}$ . Samples of the cuttings used in these experiments were examined for starch when collected. Those cuttings which rooted contained considerable reserve starch. In experiments with summer canes, as well as with overwintered ones, there seemed to be a correlation between starch content and rooting behavior.

So far, no differences in structure of the two roses which could clearly account for their difference in rooting behavior have been detected. American pillar canes are thicker and stiffer than Dorothy Perkins canes. This is due largely to the greater amount of xylem in American pillar. The pith of Dorothy Perkins is more compact than that of American pillar.

TABLE II  
ROOTING OF DOROTHY PERKINS AND AMERICAN PILLAR ROSE CUTTINGS AT CONSTANT TEMPERATURES

	TEMPERATURE										CONTROL IN LIGHT ROOM TEMPERATURE	
	32° C.		25° C.		20° C.		15° C.		10° C.		5° C.	
	No. of cuttings	No. rooted	No. of cuttings	No. rooted	No. of cuttings	No. rooted	No. of cuttings	No. rooted	No. of cuttings	No. rooted	No. of cuttings	No. rooted
Experiment 1, December 15-January 12												
American pillar... Dorothy Perkins.	9	0	9	2	9	2	.....	.....	9	0	.....	3
	6	0	6	6	6	6	.....	.....	6	3	.....	6
Experiment 2, January 15-February 21												
American pillar... Dorothy Perkins.	9	0	10	0	9	0	12	1	10	0	12	0
	10	0	11	1	9	8	13	12	12	9	12	5

The branches are similar in structure during their early development, but after three or four days the cambium at the base of Dorothy Perkins branches becomes unusually active toward the outside, and produces an extensive region of parenchymatous phloem tissue. The adventitious roots originate in this tissue.

### Discussion

New shoots on the overwintered canes of Dorothy Perkins rose send out roots while in air of high humidity, as well as when planted in sand or peat moss. Moisture, therefore, must be the primary factor which induces root growth in Dorothy Perkins if the temperature and oxygen supply are favorable. Rooting occurred throughout a wide range of temperature, and light did not seem to play a part. Layered canes rooted as well as cuttings; evidently separation from the mother plant was not an important factor.

On the other hand, new shoots arising from overwintered canes of American pillar rose did not root. Some factor or factors other than moisture must be involved with this species. The primary difference detected between the two roses was in the reserve starch storage. Dorothy Perkins contained much more starch than American pillar in the two successive spring seasons. Winter canes kept in saturated atmosphere send out new shoots which necessarily draw on the stored food supply. The starch first disappeared from the side opposite the bud, and finally the last trace was found at the base of the new shoot. American pillar rose exhausted its entire supply in 10-12 days, whereas Dorothy Perkins rose showed a moderate amount after three to four weeks. In a few cases American pillar rooted in the fall when its starch content was high, and both failed to root when they lacked starch reserve.

Preliminary experiments to increase the carbohydrate content of American pillar canes and to decrease it in Dorothy Perkins before testing their rooting have not yet been successful.

The relation between the reserves and the rooting behavior of cuttings has been reported by other investigators. KRAUS and KRAYBILL (3) found that cuttings of tomato which are old and yellow in color, high in carbohydrates, and low in total nitrogen and nitrates, root well in humid air. Green stems which contain starch



and are fairly high in total nitrogen will root, but not so profusely as the former; and green stems without starch reserves and very low in free reducing substances but high in total nitrogen and nitrate nitrogen will not root. This work was extended and confirmed by STARRING (13), REID (7, 8, 9), and SCHRADER (10), all working with tomato.

PRIESTLEY (6) states that in some cases the food supply largely controls the production of new root initials. He thinks that adventitious roots arise in the cambium, and that stems which have no true endodermis allow leakage of food out through the cortex to the superficial meristems, with consequently not enough food remaining in the cambium for the production of root initials. Some etiolated shoots produce an endodermis. These make little growth from the superficial meristems, but root with ease, since the nutrients are held by the endodermis and can be used by the cambium.

SMITH (12), working with *Coleus*, found that the condition of the carbohydrate reserves had a marked influence on rooting. Plants in full light, with reserve carbohydrates largely in the form of starch, rooted sooner and better than plants in the shaded greenhouse where reserves were mostly reducing sugars.

Rooting from the bases of Dorothy Perkins branches involves, first, a special activity of the cambium by which an extraordinary amount of phloem tissue is produced, and second, the change in certain cells of the new tissue from the parenchymatous to the meristematic condition, thus initiating root primordia. The principles involved in these processes are still largely a matter of theory. PEARSALL and PRIESTLEY (5) account for the transformation of tissues from a vacuolated, non-dividing condition into a non-vacuolated, or only slightly vacuolated, meristematic condition by the fact that they lie across a gradient of hydrogen-ion concentration. Along this gradient the principal proteins of some cells are at their iso-electric point. These cells will lose water, accumulate protoplasm, and become meristematic.

SMITH (11) states:

Cell division is the expression of a series of catenary reactions depending upon the presence in a certain concentration and a certain ratio of carbohydrate and amino-radicles. Only when this carbon-nitrogen balance is maintained can



a cell remain meristematic. Under normal conditions the carbohydrate is always in excess, and it is this excess which determines cell-maturity and the cessation of new growth. If by any means the required C:N ratio can be restored, a mature tissue can be incited to regeneration.

### Summary

1. Adventitious roots are produced in humid air on the new shoots from cuttings of Dorothy Perkins rose and not from like portions of American pillar rose.

2. Microchemical examination showed that Dorothy Perkins cuttings contained more reserve starch than American pillar cuttings. At the time of rooting, Dorothy Perkins had considerable reserve starch remaining, while during a similar period the reserve starch of American pillar was depleted.

3. The changes in the reserves were the same in both roses. As the starch in the canes was hydrolyzed, the content of reducing sugars increased, especially in the new branches. Asparagin was abundant in the branches after 8-11 days.

4. Only slight differences in the anatomy of the overwintered canes were noted. A swelling at the base of new branches of Dorothy Perkins was produced by an unusual development of secondary phloem. Adventitious roots were initiated in this region. These changes did not occur in American pillar branches.

5. Rooting from the bases of young shoots of Dorothy Perkins rose seemed to be related to the high content of reserve starch.

NORTHWESTERN UNIVERSITY  
EVANSTON, ILL.

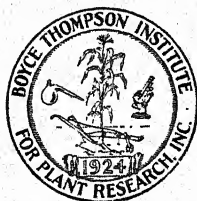
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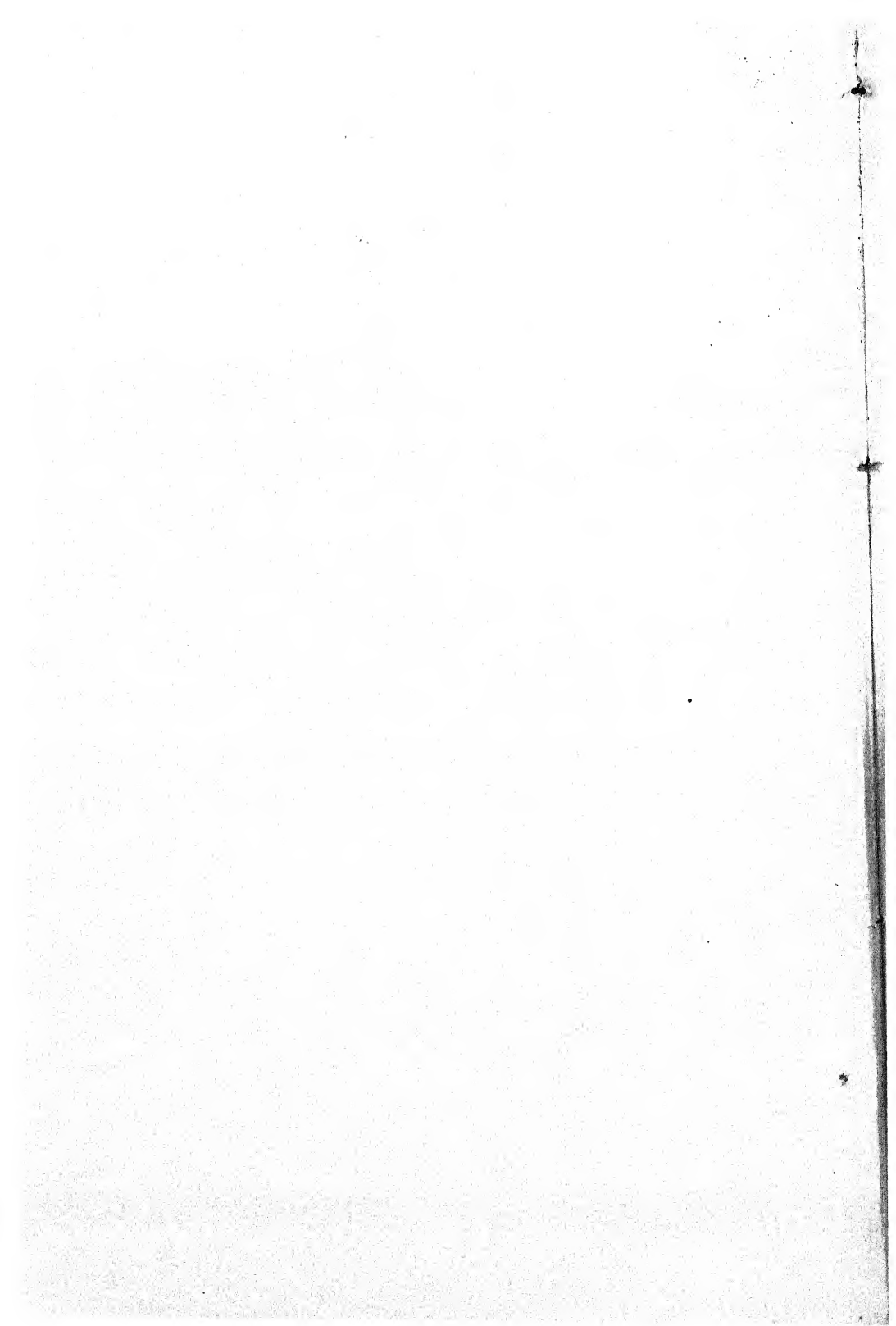
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